PREVALENCE OF ESBL PRODUCING GRAM NEGATIVE BACILLI IN POST OPERATIVE WOUND INFECTIONS

Dissertation Submitted to

Coimbatore medical College for

M.D. DEGREE in Microbiology

Branch IV

The Tamil Nadu

Dr. M.G.R. Medical University

Chennai

September– 2006

CERTIFICATE

This is to certify that this dissertation work entitled **"PREVALENCE OF ESBL PRODUCING GRAM NEGATIVE BACILLI IN POST OPERATIVE WOUND INFECTIONS"** is a bonafide record of work done by **Dr. J. VASUDEVAN** in the department of Microbiology, Coimbatore Medical College and Hospital, Coimbatore - 641 004 under the effective guidance of **Dr. R.K. GEETHA M.D., D.C.P.,** Professor and HOD of Microbiology during the period of study (2003-2006).

DEAN **GUIDE**

DECLARATION

I solemnly declare that the dissertation titled **"PREVALENCE OF ESBL PRODUCING GRAM NEGATIVE BACILLI IN POST OPERATIVE WOUND INFECTIONS"** was done by me at Coimbatore Medical College Hospital during the period from October 2004 – September 2005 under the guidance and supervision of Professor **Dr. R.K. GEETHA M.D., D.C.P.,** Professor and HOD of Microbiology, Coimbatore Medical College, Coimbatore.

This dissertation is submitted to the Tamilnadu Dr. M.G.R. Medical University towards the partial fulfillment of the requirement for the award of M.D. Degree (Branch – IV) in Micro Biology.

Place:

Date: **Dr. J. VASUDEVAN**

acknowledgement

ACKNOWLEDGEMENT

I extremely thankful **Dr. T.P. KALANITI, M.D.,** our beloved Dean, for allowing me to utilize the Hospital facilities for doing this study.

I express my heartfelt thanks and deep sense of gratitude to Prof. **Dr. R.K. GEETHA, M.D. D.C.P.,** Professor and H.O.D. of Microbiology for their whole hearted guidance and immense help given to me.

I take this opportunity to express my sincere thanks to **Prof. Dr. ANBU. N. ARAVAZHI M.D.,** for his constant encouragement and continuous support throughout the period of this work.

I am ever grateful to **Prof. Dr. K. RAJENDRAN M.D.,** for his valuable guidance and suggestions given to me.

I wish to extended my special thanks to **Dr. M. BASKAR M.D.,** Asst. Prof. Department of Microbiology, Coimbatore Medical College for guiding me throughout the period of study with constant encouragement and advice.

My sincere thanks to **Mrs. SELVANAYAKI** and **Mr. MURUGANANTHAM,** Laboratory Technicians for their help in doing all investigations.

Last but not the least I would like to thank my **COLLEAGUES** and all other in the department for their kind co-operation.

I bestow my worship to Almighty and my Parents for their blessings showered on me.

CONTENTS

S. No.		Page No.
1.	INTRODUCTION	1
2.	AIMS AND OBJECTIVE	3
3.	REVIEW OF LITERATURE	4
4.	MATERIALS AND METHODS	35
5.	RESULTS	41
6.	DISCUSSION	56
7.	CONCLUSION	62
8.	SUMMARY	65
	ANNEXURE	
	BIBLIOGRAPHY	
	APPENDIX	

INTRODUCTION

PREVALANCE OF ESBL PRODUCING GRAM NEGATIVE BACILLI IN POST OPERATIVE WOUND INFECTION

INTRODUCTION

Resistant bacteria are emerging world wide as a threat to the favorable out come of common infections in community and hospital settings. Even before the development of penicillin the first beta lactam anti biotic there was an emergence of resistance to betalactam antibiotics (Ref.30). The most important single mechanism of resistance to these antibiotics is due to the production of beta lactamases by gram positive and gram-negative organisms. The enzymes are thought to have evolved from penicillin binding proteins with which they show some sequence homology. This development was likely due to the selective pressure by beta lactam producing soil organisms found in the environment. Because of their increased spectrum of activity against the oxyimino-cephalosporins, these enzymes are called extended spectrum beta lactamases which are capable of hydrolyzing and inactivating a wide variety of beta lactam antibiotics like third generation cephalosporin's and penicillin's. ESBL producing gram negative bacteria are of

increasing importance on a worldwide basis and these pathogens are beginning to pose a serious threat. There is some evidence to suggest that over use of β lactams has imposed a selective pressure on pathogens to acquire resistance genes and mutate these to confers a broader range of activities (Ref. 34). ESBLs have been reported from all parts of the world. However there is wide variation of the prevalence even in closely related regions. ESBLs have been found in a wide range of gram-negative bacteria and the majority of the strains belong to the family Enterobacteriacae. Enterobacteriacae producing ESBLs enzymes are a clinical threat and have been associated with increased mortality in severe infection.

This study was undertaken to determine the prevalence of ESBL producing gram negative bacilli in post operative wound infections and also importance of detection of these enzymes and their epidemiology.

AIMS AND OBJECTIVES

AIM AND OBJECTIVES

1. Collection of specimens from post operative wound infections.
2. Inoculation of specimens onto suitable culture media for isolation of organisms.
3. Selection of gram negative bacilli that are resistant to third generation cephalosporins.
4. Detection of ESBL from these strains using screening (Double Disk Synergy Test - DDST) and confirmatory test (Phenotypic Confirmatory Disk Diffusion Test)
5. Analysis and comparison of different methods for ESBL detection.
6. To study the prevalence of ESBL producing Gram negative bacilli in post operative wound infections.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Penicilins and cephalosporins are called β lactam antibiotics as they posses β lactam ring in their structure. Bacteria acquired resistance to these antibiotics by production of enzymes called **β lactamases** which hydrolyse the β lactam ring. Extended spectrum β lactams are those antibiotics which are not affected by common β lactamases. Enzymes which confer resistance to these oxy imino β lactams and mono bactams are called Extended spectrum β lactamases (ESBL). These are derivatives of common β lactamases that have under gone one or more amino acid substitution(s) near the active site of the enzyme thus increasing their affinity and the hydrolytic activity against third generation cephalosporins and monobactams.

The ESBL enzymes are plasmid mediated enzymes capable of hydrolyzing and inactivating a wide variety of β lactams – third generation cephalosoporins, penicillins and aztreonam. These enzymes are the result of mutations of TEM 1 and TEM 2 and SHV1. All of these β lactamase enzymes are commonly found in the family Enterobacteriacei TEM 1, TEM 2 and SHV1 enzymes confer high level resistance to first generation cephalosoporins. Widespread use of third

generation cephalosoporins and aztreonam may be the major cause of mutations in these enzymes that has led to the emergence of ESBLs. The first ESBL isolates were discovered in Western Europe in mid 1980's and subsequently in the US in the late 1980's. The resistant organisms are now a worldwide problem. The majority of ESBL producing strains are Klebsiella pneumoniae, K oxytoca, and Escherichia coli. Other enzymes in which the frequency of ESBL production is low were; Enterobacter.spp, salmonella.spp, morganella.morgani, protus mirabillis, Serratia.marcescen and Pseudomonas.aeruginosa.

Structure of β lactamase and mechanism of action

All ESBLs have serine at their active sites except for a small but rapidly growing group of metallo β lactamases belonging to class B. They share several highly conserved aminoacid sequences with Pencillin Binding Proteins (PBPs). β lactamases attack the amide bond in the β lactam ring of penicillin and cephalosoporins with subsequent production of pencillinoic acid and cephalosoporic acid, ultimately rendering compounds antibacterially inactive.

Classification Schemes

Various classification schemes have been proposed by many researchers. Classification of Sawai et al in 1986 was based on response to anti sera. Amblers molecular class A – majority of ESBLs contain a serine at the active site and belong to these class characterized by a molecular mass of approximately 29,000 Da. Richmond and Sykes scheme in 1973 was on the basis of substrate profile. Extension of the Richmond and Sykes scheme by Sykes and Mathew in 1976 was based on differentiation by Isoelectric Focusing. In the scheme proposed by Mitsuhashi and Inoue in 1981 the category "cefuroxime hydrolyzing β lactamases" was added to "pencillinase and cephalosoporinase" classification. Recently any clasxsificaiton scheme has been developed to integrate functional and molecular characteristic. The Bush Jacoby, Medeiros Scheme puts 178 β lactamases from naturally occurring bacterial isolates into four groups based on substrate and inhibited profiles.

β lactam Antibiotics

Of all the antibiotics, β lactam antibiotics are among the most useful and frequently prescribed. They are so called due to the presence of a core β lactam ring in their structure. Pencillins and cephalosporins

will come under this group. Mechanism of action : Inhibition of synthesis of bacterial peptidoglycan cell wall. Cephalosporins : First generation – having G +ve and modest G -ve activity. Second Generation : Having some what better activity against G –ve bacteria and some with anti anaerobe activity. Third Generation : Less activity against G +ve organisms but much more activity against the Enterobacteriaceae. Fourth Generation : Spectrum similar to the third but having increased stability to hydrolysis by β lactamases. Combination of β lactam antibiotics with β lactamase inhibitors – sulbactam/ cefoperezone combination is an effective and safe alternative to Aminoglycsoide / clindamycin combination for intra abdominal infections. SHINAGAWA et al – studied the efficacy and safety of sulbactam/ cefoperezone in the treatment of billary tract infections. It had an efficacy of 79.9% with the efficacy in patients with cholecystits, cholangits and liver abscess at 89%, 77.3% and 21.4%.

IAKOLEV et al – studied the efficacy of sulbactam / cefoperezone in wound infections. The clinical efficacy was 92% and the bacteriological efficacy was 76%

TABLE: Correspondence between Ambler's molecular classification of β-lactamases and the functional groups of Bush et al.[a]

Structural class (Ambler)	Functional group (Bush)	Activity[b]							Inhibition by clavulanate
		Penicillin	Carbenicillin	Oxacillin	Cephaloridine	Cefotaxime	Aztreonam	Imipenem	
Serine β-lactamases	2a	+++	+	-	±	-	-	-	++
A	2b	+++	+	+	++	-	-	-	++
	2be	+++	+	+	++	++	++	-	++
	2br	+++	+	+	+	-	-	-	-
	2c	++	+++	+	+	-	-	-	+
	2e	++	++	-	++	++	++	-	++
	2f	++	+	?	+	+	++	++	+
C	1	++	±	Inhibitor	+++	+	Inhibitor	-	-
D	2d	++	+	+++	+	-	-	-	±
Undetermined[c]	4[c]	++	++	++	V	V	-	-	-
Zinc β-lactamases									
B	3	++	++	++	++	++	-	++	-

[a]Data from Ambler's classification and the classification of Bush et al.(Ambler, 1980; Bush et al., 1995) This table includes some simplifications. In particular,

Group 2d includes

(i) Molecular class A oxacillinases from *Actinomadura* and *Streptomyces* spp., as well as class D enzymes from gram-negative rods; (ii) hydrolytic activity varies within each group, and (iii) sequences remain to be determined for many enzymes included in Bush's scheme.

[b] +++, preferred substrate (highest Vmax); ++, good substrate; +, hydrolyzed; ±, barely hydrolyzed; -, stable; V, variable within group; ?, uncertain.

[c] None of Bush's group 4 enzymes has yet been sequenced; they are assumed to "l?e serine types because they lack carbapenemase activity.

Extended spectrum beta lactamases

SENTRY Antimicrobial
Surveillance Program 1997-9

- probably underestimated. Significant proportion of laboratories do not perform tests specifically designed to detect ESBLs
- increasing
- considerable geographical variation.

■ Klebsiella pneumoniae ■ E. coli ■ Proteus mirabilis

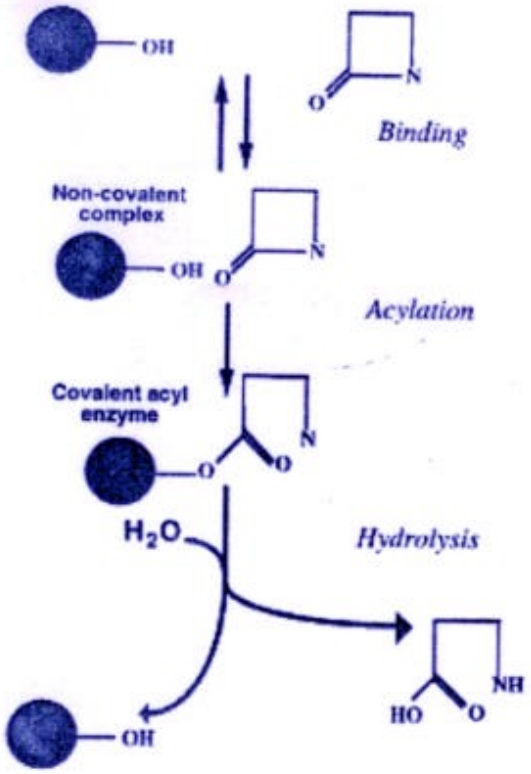


Fig. 1. Action of a serine β-lactamase.

The enzyme first associates noncovalently with the antibiotic to yield the noncovalent Michaelis complex. The β-lactam ring is then attacked by the free hydroxyl on the side chain of a serine residue at the active site of the enzyme, yielding a covalent acyl ester. Hydrolysis of the ester finally liberates active enzyme and the hydrolyzed, inactive drug. This mechanism is followed by β-lactamases of molecular classes A, C, and D, but class B enzymes utilize a zinc ion to attack the β-lactam ring. (Livermore, 1995)

Types Of ESBLs

Most are derivatives of TEM or SHV enzymes . there are now > 90 TEM type N beta lactamases and > 25 SHV type Beta lactamases. They are most often found in E.coli and K.pneumoniae also found in proteus spp. Providencia spp. With both of these groups of enzymes a few point mutations at selected loci with in the gene give rise to the extended spectrum phenotype. **TEM-1** : First plasmid mediated B lactamases in gram negatives described in the 1960's found in a single strain of E.coli isolated from a blood culture from a patient Temoniera in Greece which is able to hydrolyze penicillin and early cephalosporins such as cephalothin and cephaloridine. **TEM-2:** First derivative of TEM-1 had a single amino acid substitution from the original Blactmases, caused a shift in the isoelectric point from a PI of 5.4 to 5.6 but did not change the substrate profile. **TEM-3:** Reported in 1989 first TEM type B lactam ase that displayed the ESBL phenotype. SHV type Blactmase – another common plasmid mediated b lactam ase found in K.penumnoiae, E.coli is SHV-(sulphhydryl) variable. **Inhibitor Resistant TEM B.Lactamases (IRT) – B.Lactamases :** In early 1990's B.Lactmases that were resistant to inhibition by clavulanic acid were discovered which were variants of TEM-1 or TEM-2 revealed by nucleotide sequencing. Inhibitor Resistant TEM B.Lactamases (IRT) found mainly in clinical isolates of E.coli but also in some strains of K.pneumoniae, K oxytoca,

P.mirabilis, citrobater.freundii. Although Inhibitor Resistant TEM B.Lactamases (IRT) variants are resistant to inhibition by clavulanic acid and sulbactan, there by showing resistance to the B.Lactamase –inhibitor combination , they remain susceptibile to inhibition by tazobactam and subsequently the combination of piperacillin-tazobactam combination. CTXM : In recent years a new family of plasmid mediated ESBLS called CTXM that preferentially hydrolyze cefotaxime mainlyfound in strains of slamonella enterica serovar.Typhimurium & E.coli. they included : CTXM1 (Germany, Italy)M2 (Argentina) M3(Poland), M4(Russia),M6,M7 Greece), M5(Latvia), M8 (Brazil),M9,10(Spain).Tohoenzymes 1,2 prevalence in Japan. OXA: type enzymes are another growing family of ESNBLS belong to molecular class D and functional 2d, confer resistance to ampicillin and cephalothin, has high hydrolytic activity against oxacillin and cloxacillin, poorly inhibited by clavulanic acid. OXA type ESBLS are found mainly in P.aeruginosa. OXA 11 14 15 16 17 (Turkey) OXA 13 (France) 18 19 28(France).

Pathogenesis of wound infection

Wound infection has probably always been a major complication of surgery and Trauma. Bacterial Contamination is inevitable but controlled by strict adherence to principles of asepsis.

Surgical incision itself – an initial act – which by breaching the skin disrupts the primary barrier to infection. Then microorganisms may gain access to the blood stream and deep tissues through the incision. Dead space may result with increased risk of infection. Areas of tissue ischaemia, necrosis and inadequate blood flow are created – predisposing to the formation of exudates and haematomas.

Types Of Post Surgical Wounds

South ampton wound grading system

GRADE	
0	Normal Healing
I	Normal Healing with mild bruising or erythema
Ia	Some bruising
Ib	Considerable bruising
Ic	Mild erythema
II	Erythema + other signs of inflamation
IIa	At one point
IIb	Around sutures
IIc	Along wound
IId	Around wound
III	Clear or haemoserous discharge
IIIa	At one point only ≤ 2 cms
IIIb	Along wound > 2 cms
IIIc	Large volume
IIId	Prolonged > 3 days
Major complications IV	pus
IV a	At one point only ≤ 2 cms

IVb	Along wound > 2 cms
V	Deep severe wound infections with or without tissue breakdown - Hematoma requiring aspiration

Trends of Surgical wound infection

In a study (Ref. 19) that investigated surgical wound infection rates in 47 hospitals from 1986 – 1990 –Surgical wound infection rates were :

After colon surgery = 6.8 % highest. Coronary artery bypass graft = 4.6%. Gastric surgery = 4.5%. In a comparative study of medical intensive care unit (ICU) patients and surgical ICU patients ---- Nosocomial infection rates were higher in the SICU patients (31% vs 24%). Incidence of post operative wound infection has fallen over the years ------From over 40% - for dirty wounds 1970's to less than 30% by the end of the 1980's.From 20 to 11-12% for contaminated wounds and from 9 to 7-8% for clean contaminated wounds.

Common Surgical Pathogens

In one study : 33% of all surgical infections caused by staph aureus. most common wound pathogens after intrabdominal surgery were : Enterococci, Entrobacter, Spp, E.coli.

48.4% of incisional wound isolates = Gram positive organisms

Type of operation	Anticipated Pathogen
Open heart Surgery, Vascular surgery	S.aureus, Coagulase positive staphylococcus GNB
Lower Extremity amputations	S.aureus, Coagulase positive staphylococcus GNB
Gastroduodenal	Streptococci, Bacteroides, Spp.colifoms
Billary Tract high risk	Coliforms, enterococcus
Vaginal/Abdominal hysterectomy/Cessarian Section	Group B, Strptococi, Enterococcis, anaerobes Enerobacteriaceae
Colorectal, Appendicetomy	Enerobacteriaceae, Anaerobes
Cranitomy / Lamiinectomy	S. aurues coagulase positive staphylococcus.

SSI – SURGICAL SITE INFECTION RATES BY WOULD CLASSIFICATION AND NNIS SYSTEM RISK INDEX

Incision classification	Risk index category(% infection)			
	0	1	2	3
Clean	1.0	2.3	5.4	-
Clean contaminated	2.1	4.0	9.5	-
Contaminated	-	3.4	6.8	13.2
Dirty infected	-	3.1	8.1	12.8
Over all	1.5	2.9	6.8	13.0

(Ref : John .L.Cameron current surgical therapy 8th Edition

NNIS – National Nosocomial infection surveillance. Post operative wound infections constitutes to major source of morbidity and mortality for surgical patients. Surgical wound infections divided into:

1. Incision involving tissues above facia – 60%
2. Deep (invaliding tissue at or below facia)
3. Nosocomial surgical wound infections – 40%

Variables increasing risk of post Operatives infection

Patients variables - Age over 60yrs Malnutitirtions, obesity, Diabetes mellitus, Radiation therapy, steroid therapy, malignancy, Immuno Suppressive therapy.

Preoperative variables - Duration of preoperative hospitalization, Duration of Surgery, present of hematoma, use of drains, repeat operation (during same operation), insertion of foreign body or implant.

ESBL Detection Techniques

ESBL Detection Techniques : (Ref. 30)

Clinical Micro biology Techniques

1. Standard NCCLS interpretive criteria.
2. NCCLS ESBL Confirmatory Test
3. Double Disk approximation Test

4. Three Dimensional Test
5. E-Test, ESBL Strips
6. Viteke ESBL Test

Molecular Detection Techniques

1. DNA Probes
2. PCR
3. Oligotyping
4. PCR-RFLP
5. PCR-SSCP
6. LCR
7. Nucleotide Sequencing

Several ESBL detection test are based on the KIRBY-BAUER DISK DIFFUSION Methodology

Routine antibiotic susceptibility testing methods are not capable of detecting ESBL resistance without modification. There are chances of ESBL producers likely to be reported falsely as susceptible to the cephalosporins,unless specific ESBL screening and confirmation tests are carried out.

ESBL Detection is a Two-Step Process

A screening step with an indicator cephalosporin to indicate possible ESBL production followed by a phenotypic confirmatory test. (Ref.34).

To establish the prevalence of ESBL strains in clinical isolates we have to examine the available phenotypic ESBL detection methods and to develop an optimal protocol for routine ESBL testing. Two different protocols were used : (Ref. 34)

Protocol-1 : Three test panels were used for ESBL detection using the NCCLS method. (K.pneumoniae ATCC 700603 was used as positive control for ESBL).

1. ESBL –E-TEST PANEL : Cefepime,ceftazidime,cefotaxime, each ± clavulanate.
2. CONVENTIONAL PANEL OF ESBL COMBINATION DISCS – Cefpodoxime, ceftazidime,cefotaxime each ± clavulanate.
3. EXTENDED PANEL OF ESBL COMBINATION DISCS :-

   Cefpirome (oxoid) and cefepime (MAST),each ± calvulanate.

   Cefoxtin (oxoid) disk was used for detecting AMPC production.

Protocol : 2 : Single plate disc approximation method. Four substrates cefpodoxime, ceftazidime, cefotaxime, cefepime disks were placed using a template approximately 2.5 cm from a co-amoxiclav disk in the center on a single Muller Hinton Agar Plate inoculated with a 0.5 McFarland suspension of isolate to be tested. Use of more than one substrate for screening is recommended by NCCLS. The use of Cefepime permits ESBL detection in isolates simultaneously Producing Ampc.

Double Disk Approximation Test (Ref. 30)

1. Described by Jarlier et al In this test the organism is swabbed onto a Mueller-Hinton Agar Plate. A susceptibility Disk Containing amoxicillin Clavulanate is placed in the center of the plate and Disk Containing one of the Oxyimino - β lactam antibiotics are placed 30 mm (center to center) from the amoxicillin - Clavulanate Disk, Enhancement of the Zone of Inhibition of the Oxyimino- β lactam caused by the Synergy of the Clavulalate in the amoxicillin-Clavulalate disk is a positive test. The Sensitivity of the test was increased by the reducing the distance between the disks to 20 mm in this study.(Ref : Bradford volume 14 no.4,2001)
2. Jacoboy and Han test : in which 20 micro grams of sulbactam was added to susceptibility disks containing one of the oxyimino B lactam antibiotics an increase of 5 mm in the zone of inhibition in a

disk containing sulbactam- compared to the drug alone was considered a positive test.

3. Three Dimensional test by Thomson and sanders : Following inoculation of the test organism onto the surface of a Mueller Hinton agar plate, slit is cut into the agar into which a broth suspension of the test organism is introduced. Subsequently antibiotic disks are placed on the surface of the plate 3mm from the slit.Distortion or discontinuity in the expected circular zone of inhibition is considered a positive test.
4. Phenotypic Confirmatory Double Disk Diffusion test : The NCCLS recommended the use of ceftazidime in combination with clavulanic acid. Perform the antibiogran using Muller-Hinton agar and Mcfarland 0.5 inoculum. Test both ceftazidime + clavulanic acid and ceftazidime alone. An increase in zone diameter for the combination of ceftazidime + clavulanicacid compared to ceftazidime.alone of more then >5 mm is confirmatory of the presence of ESBL.

NCCLS Screening and Confirmatory tests for ESBLS : (K.pneumoniae, K oxytoca, E.coli) (Ref .34)

Step 1 : ESBL- Screening (BP=Break point)

Disc	R. (BP.mm)	S.(BP.mm)	ESBL Screening BP(mm)
Cefpodoxime 10 mg	17	27	17
Ceftazidime 30mg	14	18	22
Aztreonam 30mg	15	22	27
Cefotaxime 30mg	14	232	27
Ceftriaxone 30mg	13	21	25

Zone diameter less than or equal to ESBL Screening BP for any one of the above antibiotics MAY indicate ESBL production. Go to Step 2.

Step 2 : ESBL Confirmatory Test

Discs	Interpretation
Ceftazidime 30 mg Ceftazidime + Clavulanic acid 30/10 mg	A 5 mm increase in zone diameter For EITHER antibiotic tested in combination with Clavulanic acid . Versus it zone when tested alone confirms ESBL production.
AND	
Cefotaxime 30 mg Cefotaxime + Clavulanic acid 30/10 mg	

Table 2 A. (Continued) Screening and Confinnatory Tests for ESBLs in Klebsiella pneumoniae, K. oxytoca, and Escherichia coli.

Method	**Initial Screen Test**	**Phenotypic Confinnatory Test**
Medium	Mueller-Hinton Agar	Mueller-Hinton Agar
Antimicrobial Disk Concentration	Cefpodoxime 10 micro g or ceftazidime 30 micro g or aztreonam 30 micro g or cefotaxime 30 micro g or ceftriaxone 30 micro g or (The use of more than one antimicrobial agent for screening improves the sensitivity of detection).	ceftazidime 30 micro g ceftazidime/clavulanic acid[a] 30/1 0 micro g land cefotaxime 30 micro g cefotaxime /clavulanic acid[a] 30/1 0 micro g (Confinnatory testing requires use of both cefotaxime and ceftazidime, along and in combination with clavulanic acid.
Inoculum	Standard disk diffusion recommendations	Standard disk diffusion recommendations
Results	Cefpodoxime zone ≤ 22 mm Ceftaz~dime zone ≤ 22 mm Aztreonam zone ≤ 27 mm· Cefotaxime zone ≤ 27 mm Ceftriaxone zone ≤ 25 mm = suspicious for ESBL production	A ≥ 5 mm increase in a zone diameter for either antimicrobial agent tested in combination with clavu1anic acid versus its zone when tested alone = ESBL (e.g., ceftazidime zone = ESBL (e.g., ceftazidime zone = 16; ceftazidime/clavulanic acid zone=21)
QC Recommendations	E. coli ATCC 25922 (see control limits in Table 3) Kleb'siella pneumomae ATCC 700603 : Cefpodoxime zone 9-16 mm Ceftazidime zone 10-18 mm Aztreonam zone 9-17 mm Cefotaxime zone 17-25 mm Ceftriaxone zone 16-24 mm	E. coli ATCC 25922 : ≤ 2 mm increase in zone diameter for antimicrobial agent tested alone versus its zone when tested in combination with clavulanic acid. Kebsiella pneumoniae ATCC 700603 : ≥ 5 mm increase in ceftazidime / clavulanic acid zone diameter; ≥ 3 - mm increase in cefotaxime / clavulanic acid zone diameter.

Controls :

E.coli ATCC 25922(-ve QC)

K.pneumoniae ATCC 700603 (+ve QC)

Quality Control : (Ref.35) The NCCLS recommends Klebsiella pneumoniae ATCC 700603 as an ESBL producing QC control(+ve control) & Escherichia.coli 25922-as a negative control in ESBL confirmation tests. zones of the cephalosporins and cephalosporins+Clavulanate disks for ESBL negative E.coli should be equal or at worst within ± 2 mm.Any greater difference implies malfunction or deterioration. This screen is than followed by a phenotypic confirmatory test that consist of determining MICs of either cefotaxime or ceftazidime with and without the presence of clavulanic acid 4 μ gm/ml.

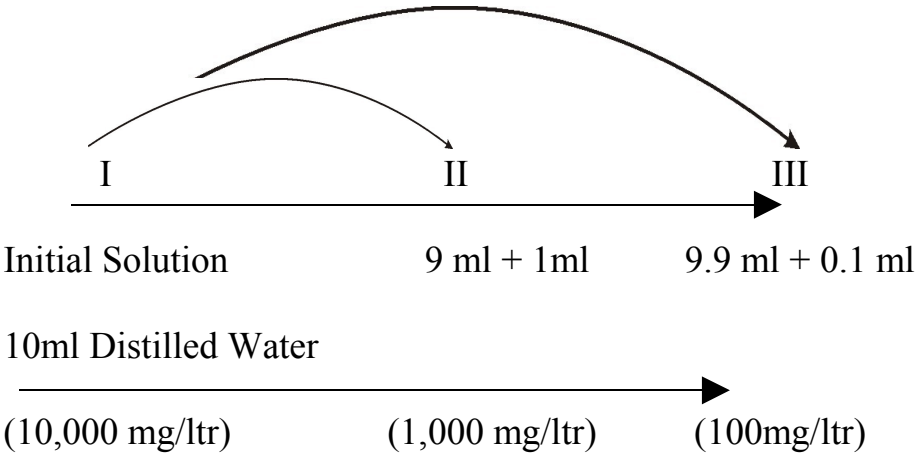
ETEST : Etest ESBL strip carries two Gradients on the one end Ceftazidime on the opposite end Ceftazidime + Clavulanic acid. MIC is interpreted as the point of intersection of the inhibition ellipse with the E test Strip edge. Ratio of Ceftazidime MIC and Ceftazidime + Clavulanic acid MIC equal to or greater than 8 indicates the presence of ESBL.

MIC Reduction Test : An 8 fold reduction in the MIC of Cephalosporin in the presence of clavulanic acid indicates – production of ESBL. (Ref .11). For MIC testing - A decrease of >3 doubling dilutions in an MIC for either Cefotaxime or Ceftazidime tested in combination with 4 μ gm /ml micro gram clavulanic acid vesus the MIC when tested alone confirms an ESBL producing Organism.(Ref.37). In one study (Ref.24) Hansotia et al where 2 micro gram per ml of clavulanic acid as been recommended. The minimum inhibitory concentrations of (MIC) of Ceftazidime, Cefotaxime and Ceftriaxone have to be determined for each of the resistant Isolates by AGAR Dilution method. The inoculum has to be standardized which is essential to provide reproducible MICs. A properly prepared Mc Farland 0.5 turbidity standard is helpful to achieve the correct working inoculum of 10^7 cfu / ml. After inoculation with a multi point inoculator, delivering 1-2 microl. The final inoculum on the agar surface will be approximately 10^4 cfu / spot. For broth dilution MICs the final Inoculum should be 10^5 cfu/ml.

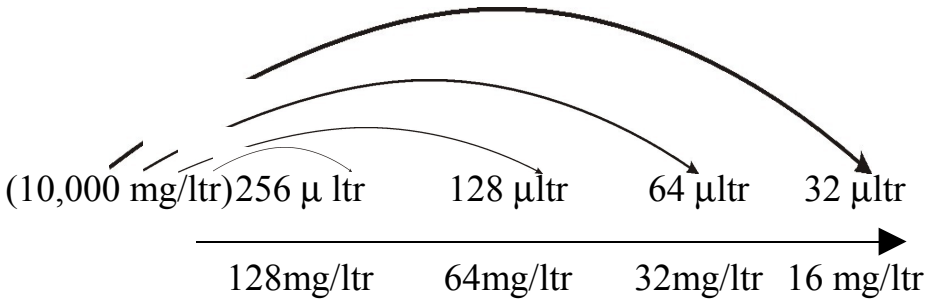
A method of preparing antibiotic dilutions for MICs Ref : Mackie & Mccartney practical medical microbiology 14th edition page162.

Three stock solutions were prepared in step 1 and used to prepare the working dilutions in steps 2 -4 before addition of agar or broth in step5.

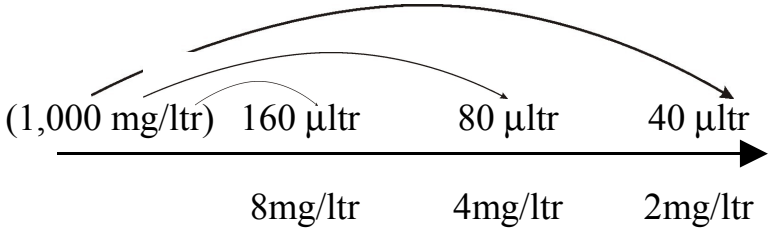
Step 1 :



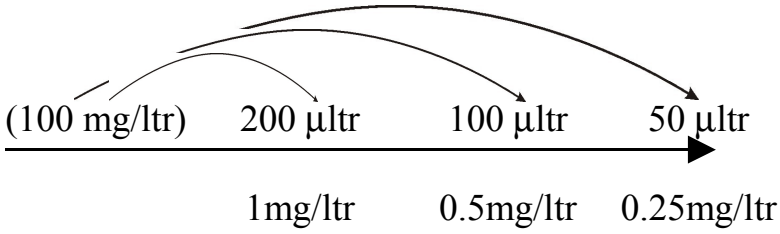
STEP 2



STEP 3



STEP 4



STEP 5

20ml agar was added to all dilution bottles including control bottle to give 10 different concentrations of each antibiotic in MHA and mixed before pouring plates or dispensing broth.

MIC Determination : (Ref.24) (Hansotia.et.al.,) The minimum inhibitory concentrations (MICs)of cefotaxime, ceftazidime has to be determined for each of the resistant isolates by Agar Dilution method using an inoculums size of 10^5 CFU/ML. Serial two fold dilutions of each test antibiotic were incorporated in Mueller Hinton Agar to give 13 or 10 different concentrations of each antibiotic in MHA. (0.03125-128 4 µ gm / ml. In one study (Ref. 8) - eight two fold dilutions of test antibiotic has been recommended.

MIC Reduction Test (Hansotia. et.al.,)

The MICs of Third Generation Cephalosporins test antibiotics determined earlier for each of the 45 resistant isolates has to be noted. Similarly the MICS of each 3 generation cephalosporins, test antibiotic combined with 4 µgm/Ml of clavulanic Acid has to be determined for each resistant isolate at different concentrations. (0.0625 – 4 µgm/Ml) of

test antibiotic in MHA. The reduction in MIC was calculated by the fractional. Inbitory concentrations (FIC) index. (Ref. 24)

$$\text{FIC Index} = \frac{MIC of\ 3GC\ \ test\ antibiotic\ \ combined\ \ with\ \ clavulanic\ Acid}{MIC\ \ of\ \ 3GC\ Test\ Antibiotic\ alone.}$$

An index of less than 0.5 was considered as evidence of synergism

II Molecular Techiques

1. DNA Probes : Early detection of B. Lactamase genes was performed using DNA probes, that were specific for TEM & SHV. enzymes. DNA sequencing has become the gold standard for analyzing novel beta lactamase genes 2. Obligo Typing - Developed by the Queltelle etal. Used to discriminate between TEM 1 and TEM 2 3. PCR – PCR with oligonucleotide primers that are specific for a betalactamase gene. Obligonucleotide primers can be chosen from sequences available in public databases such as Genbank. 4. PCR – RFLP add Restriction Fragment length prolymophism analysis to PCR. 5. PCR – SSCP – Single strand conformational polymorphism. 6. LCR : Ligase chain reaction. 7. ISO electric Forcussing Analysis. 8. PFGE – Pulse field gel electrophoresis – An epidemiological tool is used to study clonal spread and hospital outbreak.

Epidemiology

ESBLs are now a problem in hospitalized patients worldwide. The frequency of their occurrence in clinical infection is increasing globally. It Ranges from 5% In Canadian Hospitals to > 25 % in Western Pacific and > 50 % in Indian Hospitals.

The source of ESBL : Hospital or community : (Ref. 31). Community acquired outbreaks of ESBL infections are emerging due to increasing use of broad spectrum antibiotics in the community and due to large number of ESBL positive patients who carry the organisms from hospitals to community. Out breaks may be associated with inadequately disinfected equipments or contaminated surface levels – Hand Touch Sites – Hands are chief route whereby patients acquire hospital strains and hand washing programmes for staff are likely to impact on hospital acquired infection rates.

Molecular epidemiology (Ref. 36) : The mechanism of spread may be clonal strain dissemination, clonal plasmid dissemination and selection among polyclonal strains or both. The methods of transmission includes – clonal dissemination of an ESBL producer strain or the dissemination of a plasmid carrying an ESBL gene. Selective antibiotic pressure then leads to colonization of patient's skin and bowel with a risk

of subsequent infection. Thus fecal colonization may play a critical role. Out breaks may be associated with procedures – catheterization and contamination of medical devices. Through health care personal hands spread then appears to occur. Because of patient colonization, environmental contamination and hand transmission endemic strains may persists in health care settings for years. To prevent the spread and outbreak of ESBL producing microorganisms, proper infection prevention and control practices are essential.

Surveillance : Systematic method of collecting, consolidating, analyzing and distributing data with critical information on the distribution and determination of a given disease or event.

Best practices : The following practices are organized into five categories. Antibiotic stewardship – Infection control professionals play a role, Surveillance and screening, Precautions, Hand hygiene and antisepsis, Disinfection / environment.

Surveillance and screening : Preventing and controlling the spread of ESBLs is having an effective and consistent approach to surveillance. The activity of surveillance starts with micro laboratory reporting and then an assessment of patient's risk factors for colonization. Screening of

high risk target population, Neutropenic elderly patients, transplant recipients, premature neonates, post gastrointestinal surgery, prolonged extensive antibiotic use (Cephalosporins) and high risk unit admissions, ICUS, Hematology, oncology units, transplantation units, are all necessary.

The frequency of occurrence of an outbreak is dependent whether the successful clone begins to survive within hospitals and causes an outbreak. These outbreaks are often fueled by the large number of patient transfers between units & between hospitals.

Specific Risk Factors which will predispose to an outbreak were : Length of hospitals stay, time in the ICU, intubation and mechanical ventilation, urinary or arterial catheterization, prior hospitalisation, old age 60 years, Prior use of Antibiotic therapy within previous 3 months, Males, debilitated patients confined to bed, many of the patients infected with ESBLs are found in ICUs, surgical and neonatal wards.

There is a need to study the role of environment, animal sources, hospitals sludge play in the transmission of ESBLS between the species and inter and intra hospital spread. Efficient infection control practices

for containment of outbreaks – will require intervention strategies such as restriction of the use of Oxyimino- cephosporins and Antibiotic cycling.

A successful approach to the control of the spread of ESBL producing organisms involved switching to different classes of broad spectrum antibiotics. Two most successful replacement antibiotics have been imipenem and piperacillin – tazobactam (Ref. 30)

Molecular methods for studying the epidemiology of the strains involved in outbreaks are

Plasmid Profiles

Pulse field gel electro phoresis

Ribotyping

RAPD – Random Ampliphied Polimorphic DNA

AP – PCR – Arbitrarily Primed - PCR

Prevention of spread of ESBL positive organisms in the ward

Infection control precautions – barrier nursing, cohorting of patients and nurses. Contact precautions – use of disposable gloves, gowns and strict attention to handwashing. Infection control policy.Formulation of an appropriate hospital antibiotic policy will be necessary.

Co resistance to quinolones and amino glycosides are common. Usage of quinolones should be restricted as far as possible as they are strong selectors of ESBL producers.

Treatment : Treatment of ESBL producing strains of entrobactriaceae has emerged as a major challenge in hospitalized as well as in community based patients. These organisms are responsible for a variety of infections.

Urinary tract infection

Septicemia

Hospital acquired Pneumonia

Intra abdominal abscess

Brain abscess

Device related infections

Management decisions in the treatment of ESBL producers

Non antibiotic approach, Removal of the source of the infection – Removal or replacement of the foreign body or prosthetic device becomes necessary because infections of surgical implant and devices are associated with bio-film formation. Removal of a ESBL colonized intravascular line (Central venous catheter – Peripheral venous catheter) change of a colonized indwelling urinary catheter drainage of an intra

abdominal abscess removal of an infected prosthetic device, heart, valve prosthetic joint. Combination of imipenem + Amikacine was great in the treatment of life threatening infections like septicemia hospital acquired peneumonia intra visceral abscess due to faster killing rates of amikacin.

A few Beta lactams, 7 Alfa methoxy cephalosphorins – Cefoxitin, Cefotetan are effective. Beta Lactam, β lactamase inhibitor combination is very useful.Organisms such as Klebsiella pneumoniae - may become deficient in the crucial outer membrane proteins thereby rendering the Beta lactam – Beta lactamase inhibitor combination clinically in effective.

OPAT – Outpatient Parentral Antibiotic Therapy :- Intra venous administration of ertapenem and aminoglycosides (Gentamicin) once daily has given greater options in OPAT setting. (Ref. 31)

MATERIALS AND METHODS

MATERIALS AND METHODS

The study was conducted in the Department Microbiology, Coimbatore Medical College Hospital, Coimbatore from October 2004 to September 2005.

The subjects considered for our study were 120 patients admitted in the post operative surgical wards with surgical wound infections and those who had inclusive criteria of Grade IV (pus) of south ampton wound grading system. The study material consist of wound swabs from post operative surgical wound site. Samples were collected from them using the following protocol – sample collection technique - The wound area was wiped with sterile saline (or 70% alcohol) swabbed along the leading edge of the wound. Wound swabs were collected from 120 patients with clinical infected wounds and 15 from out patients attending the Review OPD (After discharge surveillance).

In each case two swabs were taken from the clinically infected wound site, on the third post operative day i.e. at the time of first change of dressing, used for culture. From the other swab smears were made gram stained and examined microscopically. After discharge surveillance swabs – two were taken one for bacterial culture and other for smear

preparation. When Gram stained smears were examined under the microscope, gram negative rods were seen (GNB). All the specimens were inoculated onto the following media. Nutrient Agar, Blood Agar, Macconkey Agar. Incubated at 37^{o}C for 24 hours. After incubation for 24 hours the plates were examined for growth and the morphology of the colonies has been observed. The gram negative bacilli colonies were tested for a panel of biochemical tests.

Based on colony morphology and a panel of biochemical tests – the organisms were identified upto the species level. The antimicrobial susceptibility of all the isolated aerobic bacterial organisms was done by KIRBY BAUER Disk diffusion method using Mueller-Hinton –Agar and the following anti microbial disks.

Amikacin 30 micro gram	Cefotaxime – 30 micro gram
Gentamicine 10 micro gram	Ceftazidime – 30 micro gram
Ciprofloxacine 5 micro gram	Ceftriaxone – 30 micro gram
Oflaxacine 5 micro gram	Co-trinaxazole 25 micro gram

Inoculum standardization : (According to NCCLS (KIRBY-BAUER))

When using the technique of KIRBY-BAUER, inoculum is standardized according to the method described by the NCCLS, which normally results in more dense growth and therefore smaller inhibition

Culture Plates (MacConkey Agar) showing Escherichia coli (LF), Klebsiella pneumoniae (MLF), Proteus mirabilis (NLF) and Pseudomonas aeruginosa in nutrient Agar (bluish green pigment production).

BIOCHEMICAL TESTS

Escherichia coli

1. Indole = +ve
2. Gulcose = +ve
3. Lactose = +ve
4. Sucrose = +ve
5. Manitol = +ve
6. Citrate utilization = - ve
7. Urease production = -ve
8. TSI = A/A = Acid slant / Acid butt and gas production
9. Methyl red test = +ve
10. Voges – proskauer test = -ve

Klebsiella pneumoniae

1. Indole = -ve
2. Gulcose = +ve (Acid and Gas)
3. Lactose = +ve
4. Sucrose = +ve
5. Manitol = +ve
6. Citrate utilization = + ve
7. Urease production = +ve
8. TSI = A/A = Acid slant / Acid butt and gas production
9. Methyl red test = -ve
10. Voges – proskauer test = +ve

Proteus mirabilis

Pseudomonas aeruginose

Escherichia coli

11. Indole = -ve
12. Gulcose = +ve (Acid and Gas)
13. Lactose = -ve
14. Sucrose = -ve
15. Manitol = -ve
16. Citrate utilization = - ve
17. Urease production = +ve
18. TSI = K/A = Alklaline slant / Acid butt, gas and H^2S production +ve

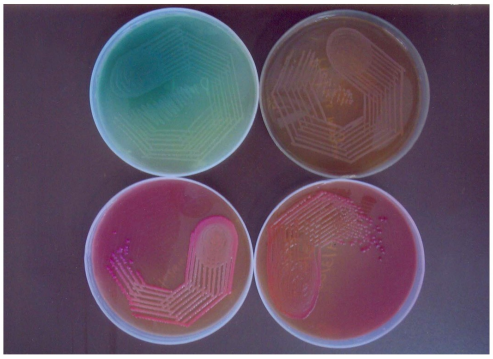
Klebsiella pneumoniae

11. Indole = -ve
12. Gulcose = Only Acid
13. Lactose = - ve
14. Sucrose = - ve
15. Manitol = - ve
16. Citrate utilization = + ve
17. Urease production = +ve
18. TSI = K/K = Alklaline slant and butt negative for H^2S and gas production.

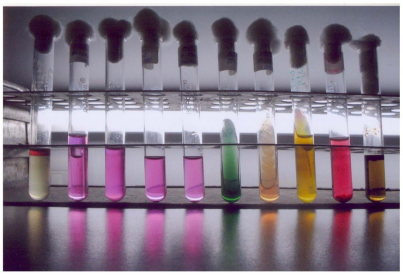
Proteus mirabilis

Pseudomonas aeruginose

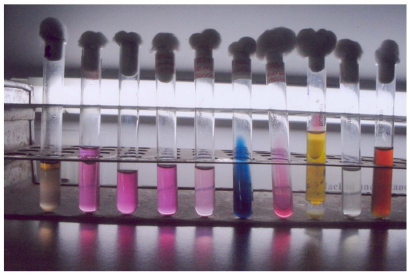
ORGANISM ISOLATED FROM WOUND SWAB



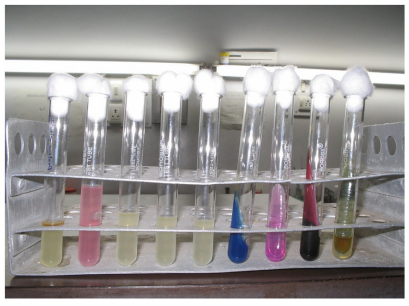
BIOCHEMICAL TESTS



ESCHERICHIA coli



KLEBSIELLA pneumoniae



PROTEUS mirabilis

Biochemical reactions of Pseudomonas aeruginosa

zones than for semi confiuent growth. Two to three colonies have been suspended into 10 ml physiological saline and thereafter diluted 0.1 ml / 10 ml. For Enterobacteriaceae and other gram negative rods 1 to 2 drops (for 9cm plate 3 to 4 drops for 14cm plate) of the final suspension have been taken and applied on to the Agar surface and distributed with a glass rod. The open plate is then dried at 35-37^{o}C for 10-15 minutes before the disks are placed onto the Agar surface.

The plates were incubated at 37^{o}c for 24 hours and the Diameter of the zone of inhibition for each antimicrobial disk was measured and recorded as resistant, intermediate and susceptible according to the standard NCCLS interpretative criteria. Those isolates which were gram –ve and showed resistance to Cefotaxime, Ceftazidime, Ceftriaxone were included in this study. 45 isolates which were gram –ve and were found to be resistant to all 3 GC antibiotics were selected for further study. All of them were screened and confirmed for ESBL production. The following methods were adopted for ESBL detection.

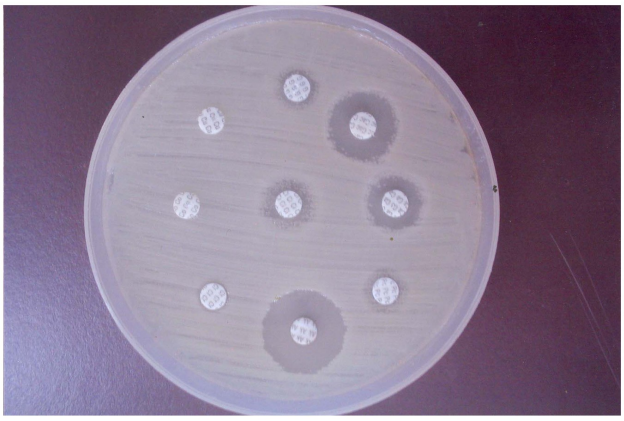
1. Screening by Double Disk approximation test
2. Confirmation by phenotypic confirmatory Double Disk Diffusion test.

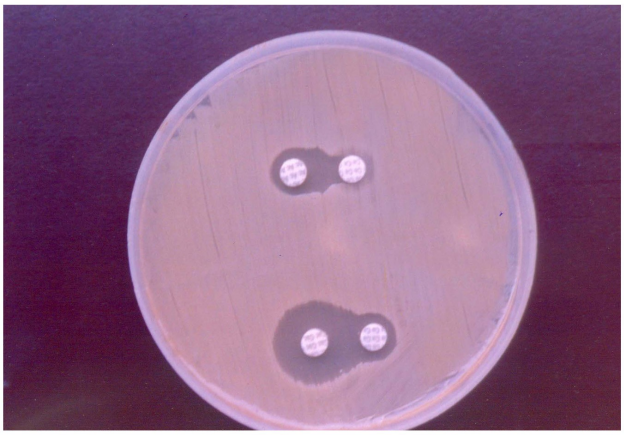
1. Double Disk approximation test : In this test the organism is swabbed onto a Mueller – Hinton Agar Plate. A susceptible disk containing

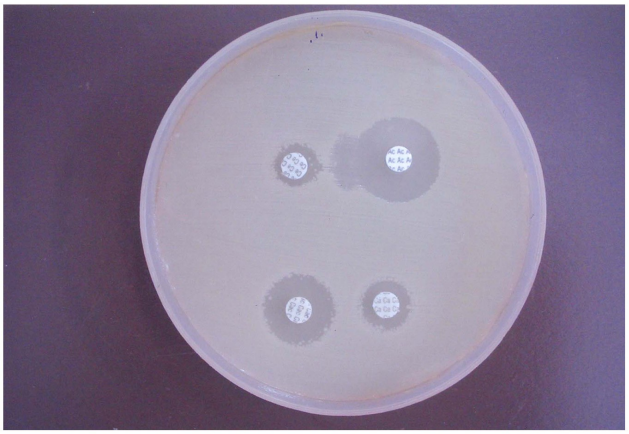
DDAT shows enhancement in the zone of cefotaxime Disk on the side facing the Augmentin indicating the production of ESBL by the isolate.

NCCLS confirmatory test – shows increase in zone diameter more than 5mm around the ceftazidime plus clavulanic acid disk compared to ceftazidime disk alone indicating the presence of ESBL in the isolate.

ANTIBIOTIC SUBCEPTIBILITY TEST & ESBL DETECTION







Amoxycillin 20µg and 10µg Clavulanic acid (Augmentin) is placed in the center of the plate. Disk containing one of the (30µg) oxyimino betalactum antibiotics are placed 30mm center to center from the Amoxycillin – Clavulanate Disk incubated at 37°C for 24 hours. It was observed that the zone size around the test antibiotic was increased towards the Augmentin Disk.

2. Confirmation by phenotypic confirmatory Double Disk Diffusion test :

This was done as per the NCCLs recommendations. On Muller Hinton Agar, Four Disks containing cefotaxime (30µg), cefotaxime / clavulanic acid (30µg / 10µg), ceftazidime (30µg), and ceftazidime / clavulanic acid (30µg / 10µg) were used. There was a > 5mm increase in zone diameter for either antimicrobial tested in combination with clavulanic acid versus its zone when tested alone, which confirmed ESBL production. ESBL was deducted in 20 isolates of Escherichia.coli and 25 isolates of Klebsiella pneumoniae (Table IV) and in none of the isolates of Proteus Spp and Pseudomonas.aeruginosa.

MIC Determination : Agar Dilution Technique – All antimicrobial agents were prepared freshly before each use to avoid any loss of potency. MHA was prepared and sterilized at 121°C for 15 minutes. The pH of each batch of MHA was checked when the medium was prepared – The medium is allowed to cool to 45-50°C in a water bath. Appropriate dilutions of antimicrobial agents were added to molten test agars. The agar and anti microbial solutions were mixed thoroughly and the mixture was poured into petri dishes to result in a agar depth of 3-4 mm.

Control plates – Drug free plates prepared from the base medium was used as growth controls.

Antimicrobial concentration and inoculum preparation – serial two fold dilutions of test antibiotic were incorporated in Muller – Hinton Agar to give 13 different concentrations of each antibiotic in MHA (0.03125-128 µgm/ml). The test inoculum was prepared with an overnight growth of each isolate, which was adjusted to a turbidity equivalent to 0.5 Mc Farland standard. Test organism was inoculated in each concentration Agar dilution plate with one µl of this suspension (10^5 CFU/ml) and the plates were incubated at 37°C. The MICs of cefotaxime, ceftazidime for

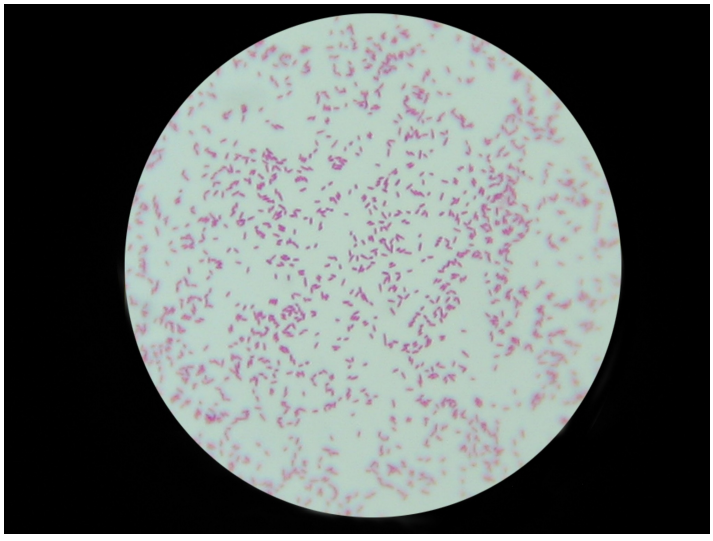
each of the resistant isolates were determined and noted. Similarly the MICs of cefotaxime, ceftazidime combined with 4µgm/ml of clavulanic

MIC of cefotaxime by Agar dilution method for ESBL producers.

1. Photograph showing cefotaxime (cef) antibiotic dilution.

2. MIC determination by Agar dilution method.

3. Photograph showing Gram Negative Rods (GNR).



acid, were also determined for each resistant isolate at different concentrations (0.0625 -4µgm/ml). It was found that there was a decrease of >3 doubling dilutions in MICs of cefotaxime, ceftazidime with clavulanic acid compared to the MICs of cefotaxime, ceftazidime.

RESULTS

RESULTS

Table -I

Sex distribution among post surgical patients in this study

Sl.No	Sex	Total Number of patients	Percentage of Post surgical patients
1	Male	76	63.33%
2	Female	44	36.67%

The total number of patients screened were 120 of which Males = 76 = 63.3%, Females = 44 = 36.67% (Table-I).

Chart -I
Sex distribution among post surgical patients in this study

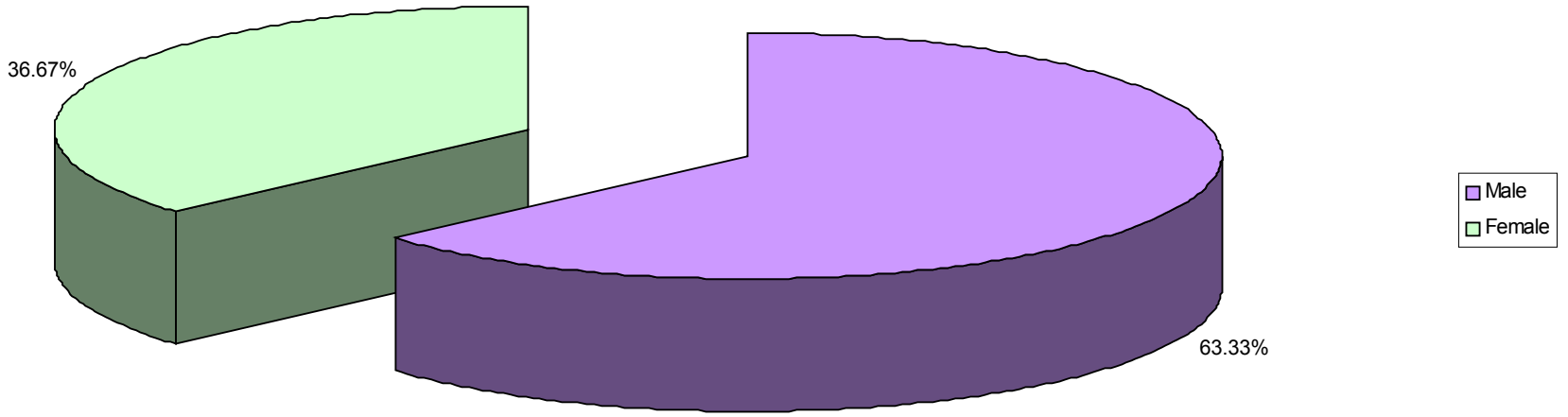


Table -II

Age distribution among post surgical patients in this study

Sl.No	Age Group	Percentage of Post surgical patients
1	25-35 years	25.4%
2	36-55 years	54.1%
3	Above 60 years	20.5%

Patients included in this study were of 25-30 years (25.4%), 36-55 years (54.1%), above 60 years (20.5%) (Table – II)

Socio Economic Status : Patients included in this study were belong to low socio economic group.

Chart -II
Age distribution among post surgical patients in this study

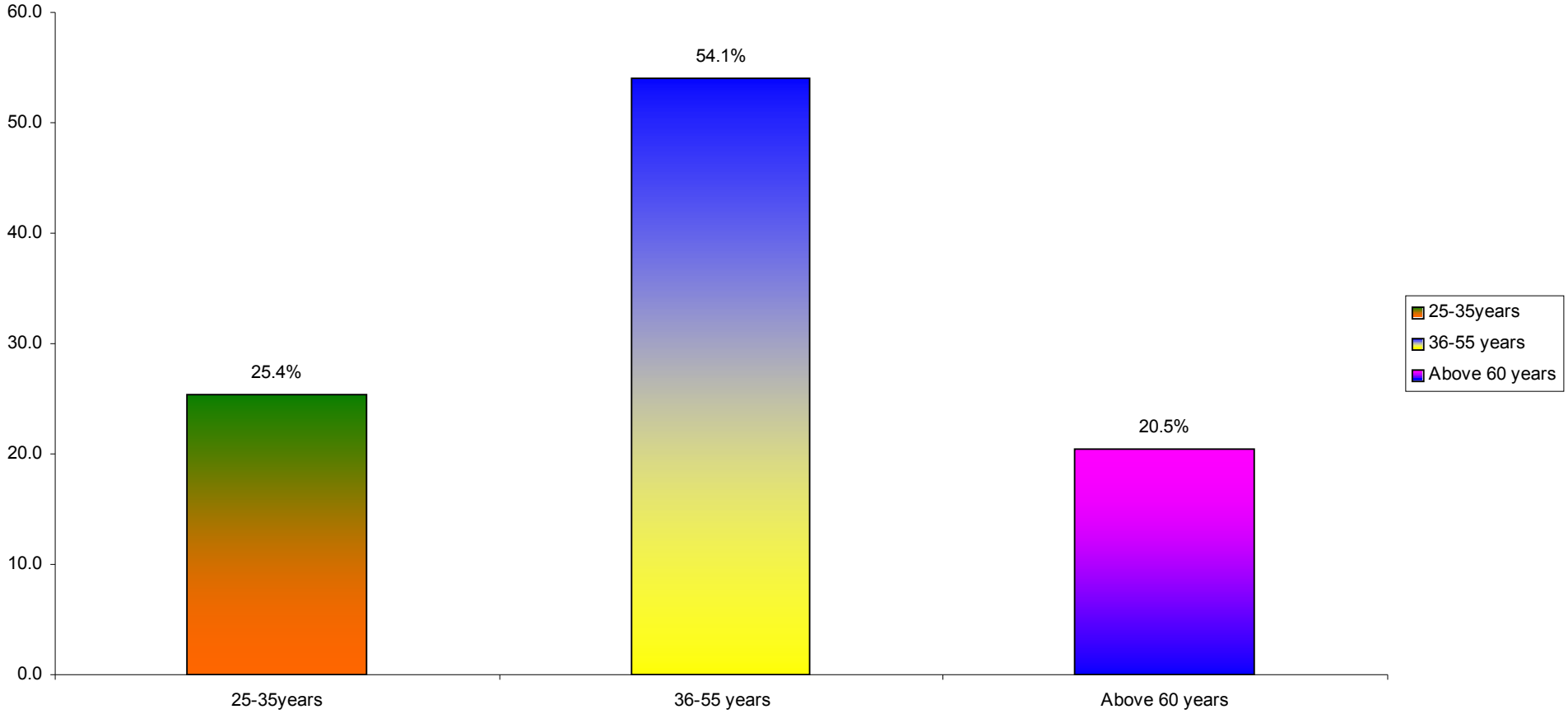


Table - III

Distribution of the various organisms isolated form from post operative surgical wound infections

Organism	Percentage
Escherichia.coli	35.83
Klebsiella.pneumoniae	43.33
Proteus spp	14.17
Pseudomonas.aeruginosa	6.67

A total of one hundred and twenty organisms were isolated. They were Escherichia.coli = 43, Klebsiella.pneumoniea = 52, Proteus.spp = 17, Pseudomonas.aeurugilosa=8 (Table-III)

Chart-III

Distribution of the various organisms isolated form from post operative surgical wound infections

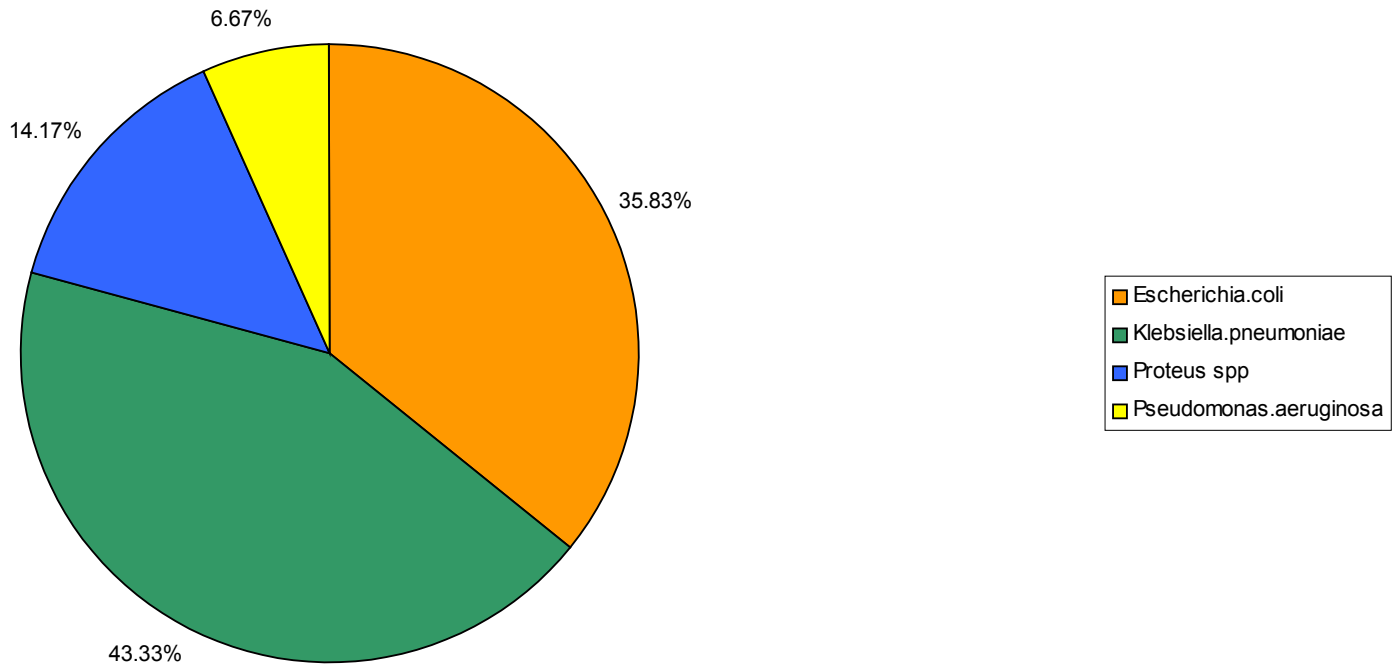


Table -IV

Distribution of ESBL +ve Strains among the different organisms isolated

Organism	Number of Organisms isolated	Number of ESBL +ve strains	Percentage of ESBL Strains
Escherichia.coli	43	20	46.50%
Klebsiella.pneumoniae	52	25	48.07%
Proteus.mirabilis	17	-	-
Pseudomonas.aeruginosa	8	-	-
Acinetobacter	-	-	-
Citrobacter	-	-	-
Enterobacter	-	-	-
Morganella morgani	-	-	-
TOTAL	120	45	37.50

Out one hundred and twenty isolates 45 were found to be ESBL producers (37.50% of the total isolates) Escherichia.coli = 20 (46.50%), Klebsiella.pneumoniea = 25 (48.07%) (Table-IV)

Char - IV
Distribution of the Various Organisms Isolated from Post Operative Surgical Wound Infections

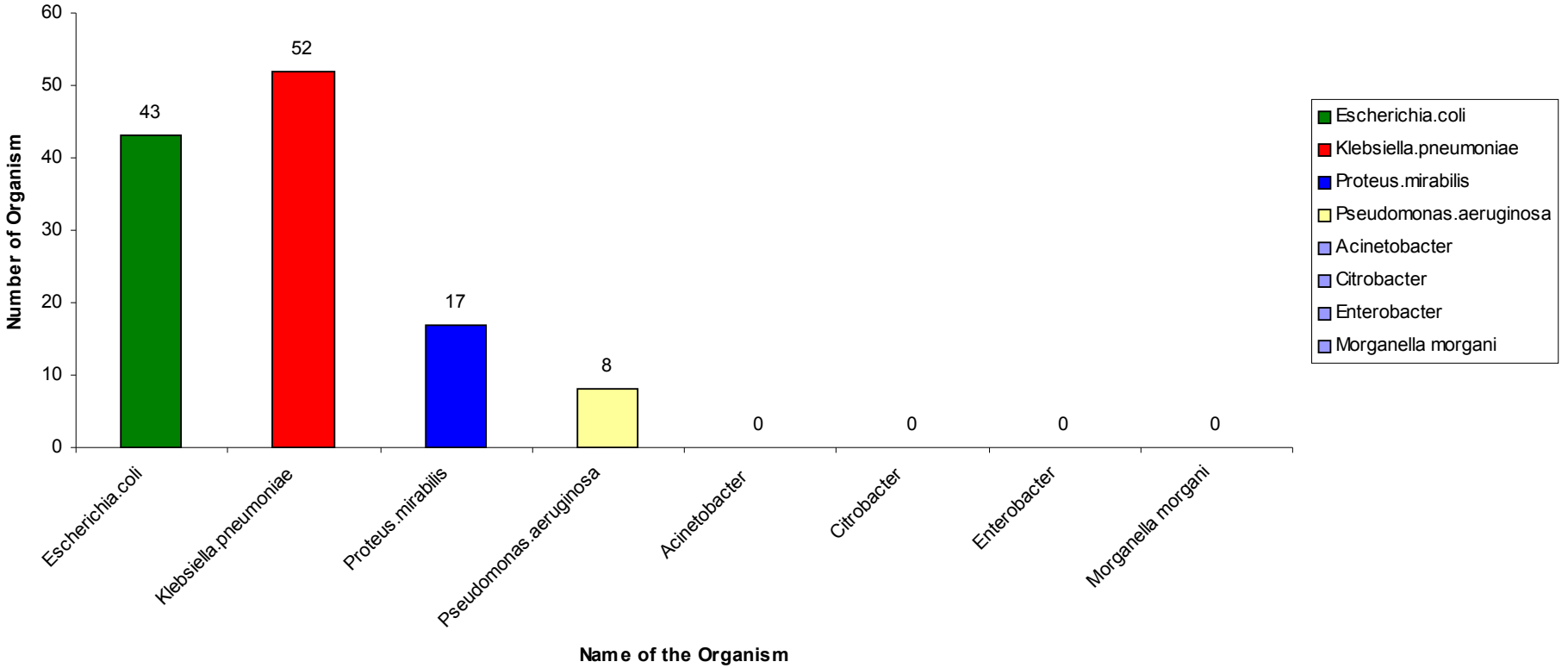


Chart - V

Number of ESBL positive isolates detected among isolates from post operative wound infections

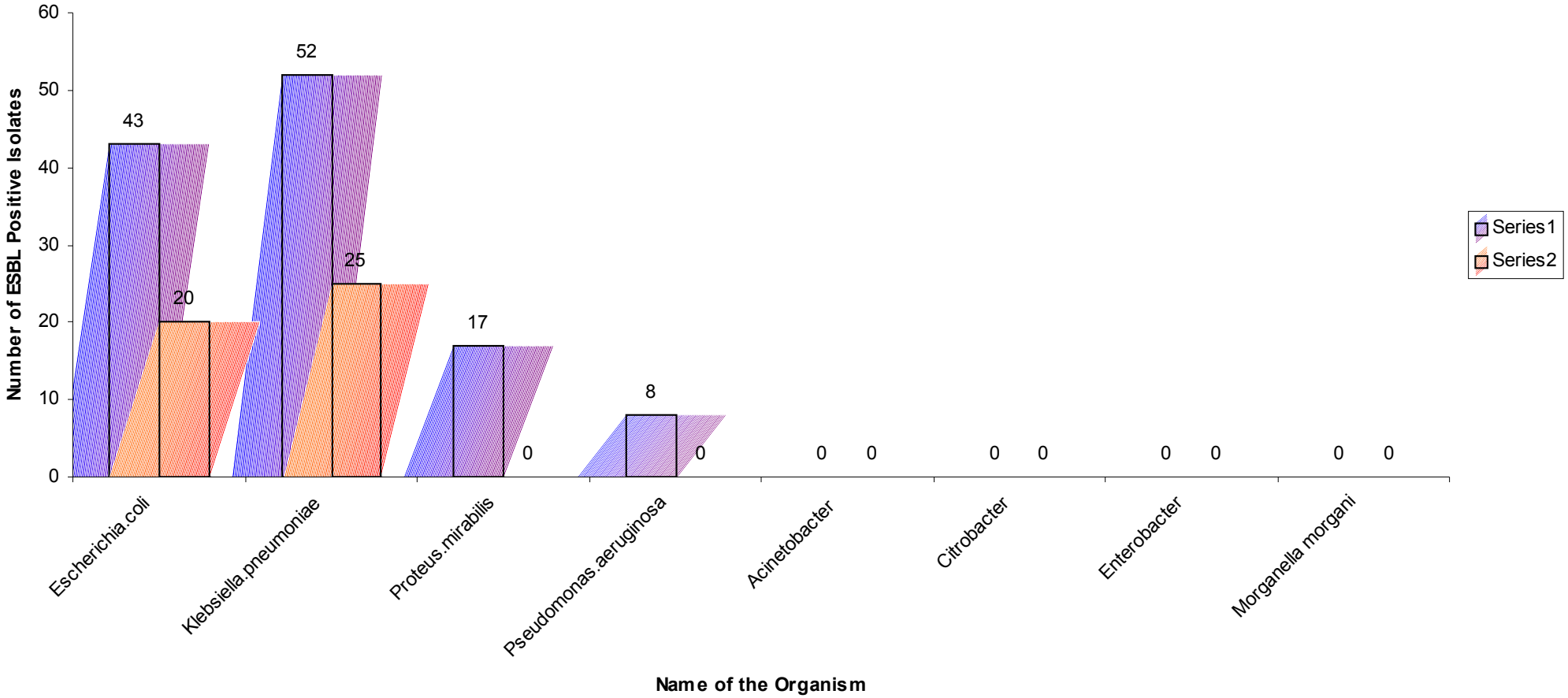
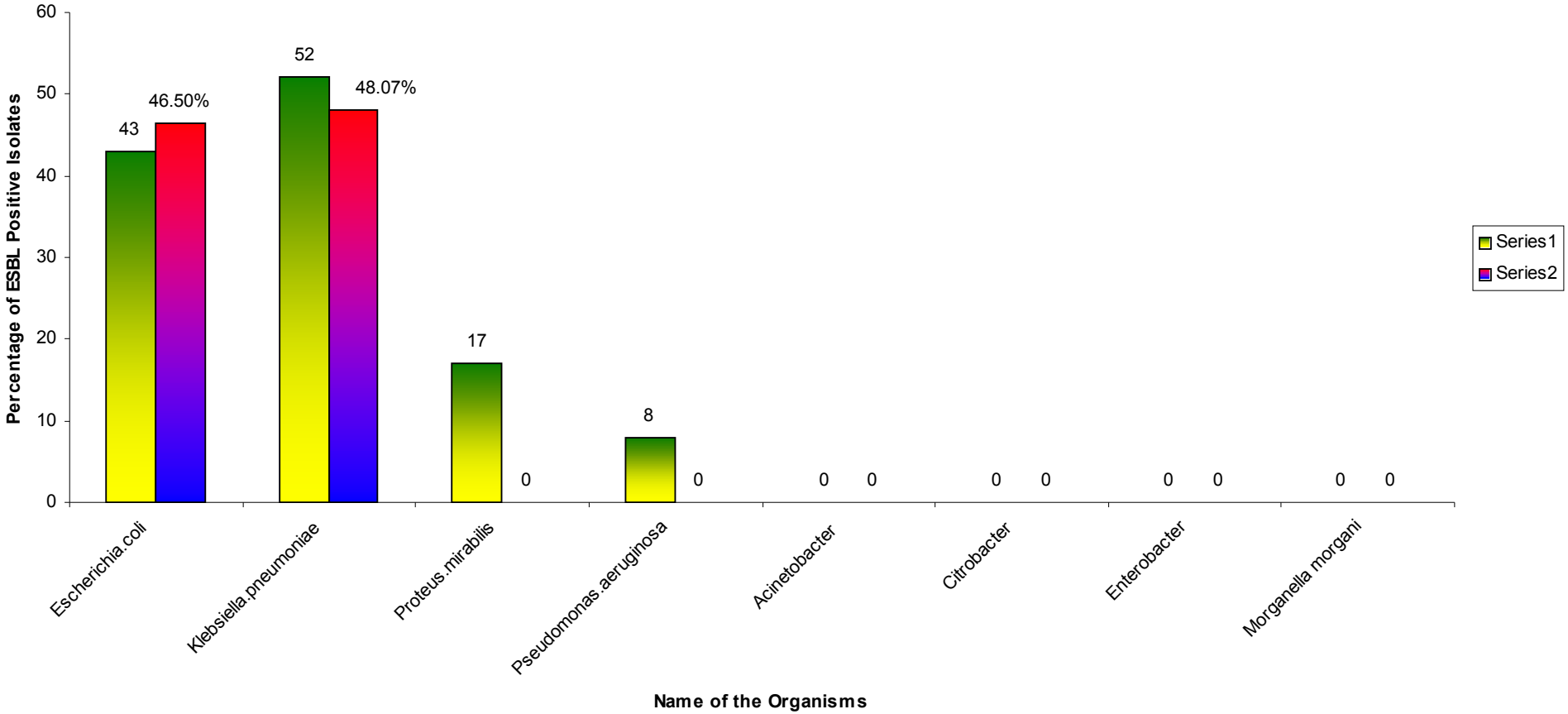


Chart - VI

Percentage of ESBL positive isolates detected among isolates from post operative wound infections



ESBL producing gram negative bacilli - screening versus confirmatory test

When the results of the initial screening tests for the two major gram negative isolates were compared with the results of the confirmatory test for ESBL test it was found that the majority of the isolates that were positive in the screening in the screening test were also positive by the confirmatory testing, although some of the isolates that were positive in the screening test were negative for ESBL productions when tested by the confirmatory method. (Table-V)

Table -V

ESBL producing gram negative bacilli - screening versus confirmatory test

Confirmatory Test Result	Isolates Positive on Screening Test		Isolates Negative on Screening Test	
	E-coli	Klebsiella Spp	E-coli	Klebsiella Spp
Positive	20 (46.50%)	25 (48.07%)	0	0
Negative	3 (6.9%)	4 (7.6%)	0	0

Table VI

Antimicrobial resistance of ESBL producing isolates to Third Generation Cephalosporins

Organism	CTAX	CTRX	CTAZ
Escherichis .coli (20)	42.17%	40.01%	41.97%
Klebsiella pneumoniae (25)	43.45%	38.83%	43.41%

Antimicrobial Resistance Pattern of ESBL producing isolates (45) to CTAX, CTRX, CTAZ, showed resistance percentage of 85.62% (CTAX), 78.84% (CTRX), 85.38% (CTAZ). In this study among the betalactum antibiotics the maximum degree of resistance was seen to ceftazidime, cefotaxime and then ceftariaxone.

Table VII

Antimicrobial resistance of ESBL producing isolates to

Non beta lactum antibiotics

Organism	OFX	Ciprofloci n	Gentamici n	Co-trimaxzone
Escherichis .coli (20)	36.77%	37.81%	36.77%	42.17%
Klebsiella pneumoniae (25)	41.60%	48.38%	40.69%	47.15%

Antimicrobial resistance of ESBL producing isolates to non beta lactum antibiotics were also studied and noted that among ESBL producing isolates (45) there was associated high level co-resistance to Co-trimaxzone (89.32%), ciprofloxcine (86.19%), oflaxacin (78.37%), and gentamicin (77.46%).

Table VIII

Antimicrbial Susceptibility Pattern of ESBL Positive Isolates

to Non Beta lactam antibiotics

Organism	R/All	S/G	S/Cip	S/AK	S/OFX	S/COTX
Escherichia.coli (20)	-	10%	5%	100%	10%	5%
Klebsiella pneumoniae (25)	-	8%	8%	88%	16%	-

Antimicrbial Susceptibility Pattern of ESBL Positive Isolates to Non Beta lactam antibiotics were also studied and found that most of them were found to be highly susceptible to amikacin, but the level of susceptibility to co-trimaxazole (5%) quinolones (cipfloxacin 13%, oflaxacin 26%) and gentamicin 18% were found to be comparatively low.

Table IX

Susceptibility Results for ESBL Positive isolates by

Agar Dilution method

Method	MIC in microgram/ml			
Agar dilution method	CTAX	CTAX + CLA	CTAZ	CTAZ + CLA
Resistant isolates ESBL producing (45)	4(8) 8(9) 16(9) 32(7) 64(7) <128(5)	0.25 0.5 >0.25 0.5 >0.5 1	2(9) 16(7) 8(10) 4(7) 32(8) 64(4)	0.125 >0.5 0.5 0.25 0.5 >0.5

A fixed concentration of 4 micro gram of clavulanic acid per ml was used in conjunction with each antimicrobial agent. Figures in parentheses = ( ) Number of isolates, CTAX = Cefotaxime, CTAZ = Ceftazidime, CLA = Clavulanic acid

The susceptibility Results for ESBL Positive isolates by Agar Dilution method (MIC determination) were studied and analysed. It was found that there was a decrease of >3 doubling dilutions in MICs of cefotaxime, ceftazidine with clavulanic acid compared to the MIC of cefotaxime, ceftazidine alone.

DISCUSSION

DISCUSSION

Of one hundred and twenty isolates – 45 were found to be ESBL producers. Which were screened and confirmed by NCCL methods. This study, showed a prevalence rate of 37.5% of the total isolates of ESBLS, among Enterobactericeae in post operative wound infection (world wide prevalence <1 to 74%). In India the prevalence rate varies from 28 to 84% whereas is the U.S it was 0 to 25% and the national average is 3% (CDCNNIS) The high percentage of ESBL producing isolates (37.5%) deducted in this study may be due to selective pressure imposed by extensive use of antimicrobials in the ICU ward and Post operative wards. The prevalence rate of ESBL detected in this study was 37.5%. But the prevalence rates deducted by both Priya Datta in a territory care hospital in India and Kanungo et al Department of Micorbiology JIPMER, were 12.6% and 20.5% respectively which were found to be lower than the value reported in the study (32,18). But the prevalence rate reported by C. Rodrigues et al was 53% which was found to be higher than the value detected in this study (28). The prevalence rate of ESBL producing Klebsiella pneumoniae detected in this study was 48.07%. But as per the studies of Hansotia et al in 1997, the prevalence of ESBL producing Klebsiella isolates was detected as 76.5% (24) in central India which was found to be higher than this study. Kanungo et al in their

studies stated that the prevalence of ESBL producing Klebsiella isolates was 43.75% (18) which was lower than the value reported in this study. The results of foreign studies have been studied by Subha et al in 2001. The incidence of ESBL producing Klebsiella isolates in the United States has been reported to the 5%. In France and England – 14 to 16% ESBL producing Klebsiella isolates have been reported. In a report from France, 24.8% ESBL positive Klebsiella pnemoniae were found in patients of more than 16 year of age. In another report from France – 3.5% of ESBL producing Klebsiella pnemoniae was obtained in patients with a mean age of 66 year. This shows that there is a wide disparity between Indian studies and Foreign studies. In this study a prevalence of ESBL producing Escherichia coli was identified as 46.50%. But Kanungo et al (18) and Anandhakrishna of JIPMER (18) in 2000 had reported incidence of 58.06% which was higher than the value of this study. Further they had reported 57.14% for ESBL producing Enetrobacter species (18). Sensitivity and Specificity of ESBL detection test – while referring the studies of Priya Datta it was found that a comparison of three test DDST, 3 Dimensional Test (3D) and IPT was made for the screening of ESBL strains (32). In their study IPT was found to be best screening method when combined with use of a ceftriaxone disk. IPT detected the maximum number of ESBL producing strains. Ceftriaxone detected the maximum ESBL rate in DDS, 3D and IPT followed by cefotaxine and

ceftazidime (32). Courdron et al found the sensitivity of ceftriaxone to be 88% ceftazidime 79% for these ESBL screening methods. Further it was confirmed by Hoe et al studies which has reported IPT was 100% sensitive and DDS was 96% sensitive (32). According to C.S. Anders – ESBL detection was made, in their study, by the comparison of 3 Dimensional Test (3D) and double disk Test. In which the 3D test provided more sensitivity of 93% of the ESBL producing strains than Double Disk Test (26). By DDST, 76.5% of Klebsiellaisolates resistant to 3G cephalosporins antibiotics was found to produce ESBLs, which was studied by Suba et al in 2002 in Central India (13). When analyzing the sensitivity of DDST it was found that Thomson Sandars has reported DDS to be 79% sensitive, Vercauteren et al found the sensitivity to be 93% and Shukla et al reported 90.6% sensitive, which were co-related with this study. Some studies questioned the sensitivity of Double Disk Test. Several modifications have been recommended including changing the distance between the Disks. A distance of 20mm center to center has been recommended by Priya Datta, Archana Thakkur in their study (32). A distance of 30 mm from center to center has been recommended by U. Chaudhry, R. Agarwal (11). The MICs of most cephalosporins rose dramatically when the inoculum of susceptibility test was raised from 10^5 to 10^7 CFU/ml. which was found by Medeiros and Crellin. It was reported by Rasheed et al that the production of that the production of

SHV–1 in a strain of K.Pnemoniae that was also lacking an Outer membrane porin protein caused a false positive in ESBL detection tests that looked at the differential between the MICs of oxyimino – B lactum antibiotics with and without clavulanate. The presence of an ESBL can also be masked by the expression of an Amp C. type enzyme in the same strain. (Ref: above).

The prevalence of ESBLs in post operative wound infection may be reduced in hospitals by instituting a hospital infection control cell, which will advise periodic Antibiotic rotation. (Every 6 months).

Apart from this there was associated resistance to other antibiotics (Table VIII). The enzymes were disseminated by the spread of either plasmids or strains, as suggested by epidemiological studies. The location of the ESBL genes on plasmids may also explain a problem of co-resistance. Such plasmid responsible for the development of Multi Drug Resistant Strains asa they may also carry co-resistance genes to aminoglycosides, quinolones, co-timaxazome. In this study prevalence of ESBL was associated with a high degree of co-resistance to the above drugs, hence there is a necessasity for using alternate antibiotics to which these organisms may be susceptible. Formulation of an appropriate Hospital Antibiotic Policy will be necessary to control this infections.

Because of selective pressure due to heavy use of expanded spectrum cephalosporins and also due to lapses in effective infection control measures, there are chances of increase of institutional out breaks. From one ESBL enzyme to another the optimal substrate profile may varies. For this reason susceptibility panels with one extended spectrum cephalosporins cannot predict resistance to other extended spectrum cephalosporins. NCCLS has recommended use of more than one substrate for screening (Ref. 32). The use of cefepime allows ESBL detection in isolates simultaneously producing Amp.c.

The findings detected in this study were co-related with risk factors like prolonged hospital stay, period of stay in SICU, urinary catheterization for post operative patients, all of which will predispose to an out break. There was empirical prescription of Ampicilli, gentamicine and metronidazole for post operative patients. Ciprofloxacin, ceftriaxone or ceftazidime or cefotaxine was added later on which will lead to prolonged hospital stay. The carbapenam antibiotics were not used at all. Further these ESBL strains will acquire co-resistance genes on plasmids.

To prevent the acquisition of these infections, precautionary measures should be taken – control of their spread by having an effective consistent approach to surveillance is important. Sensitivity and

specificity of ESBL deduction test. Even though there are merits and shortcomings of each of the detection test Double disk approximation test of Jarlier et al and the Broth Dilution MIC reduction method (NCCLS confirmatory Test) are the easiest and cost effective methods for use in clinical laboratories. However none of the detection test are 100% sensitive and specific (30).

The techniques required for the identification of the exact ESBL sub type – DNA Probing – Polymerase chain reaction, RFLP, Isoelectric focusing – are available only at research centers. Perhaps with the advent of gene chip technology in the near future – the sub type identification of ESBL and be made possible in future (32).

CONcLUSION

CONCLUSION

The prevalence of ESBL producing gram negative bacilli in operative wound infection identified in this study was 37.50% which was with in the range of 28 to 84% reported in India

ESBL production was found to be coexsisted with resistance to several other antibiotics. ESBLs are encoded by plasmids which also carry co- resistant genes for other antibiotics. We found such associated co-resistance with cotrimaxazole, 44.66% gentamicin, 38.73% ciprofloxacin, 41.09% oflaxacin 39.18%. Since co-resistance to non β lactum antibiotics, cotrimaxazole, gentamicin and quinolones was observed, combination of Imipenem+amikacin were found to be alternatives for the treatment of life threatening infections, like septicemia, hospital acquired pneumonia, intravisceral abscess due to faster killing rates of Amikacin. Our study highlights the emergence of ESBL producing Klebsiella pneumoniae (48.01%) isolates endowed with extremely wide spectrum of antibiotic resistance including resistance to cotrimaxazole, gentamicin and quinolones suggest that ESBL producing Klebsiella pneumoniae isolates have emerged as one of the major multi-drug resistance organisms. In this study, prevalence of ESBL was found

in 37.5% of the strains isolated from the surgical wounds with a high degree of associated co-resistance to non β lactum antibiotics. Therefore Formulation of an appropriate hospital antibiotic_policy is necessary to control these infections. Knowing the prevalence of ESBL positive strains in a hospital environment will become necessary to formulate a policy of empirical therapy in high risk units (Ref. 11).

Impact of post discharge surveilance of surgical infections in operative surgery is a very important factor. Surveillance feedback to surgeons is important in reducing Surgical Site Infections (SSIs) post operative infection will be missed unless post discharge surveillance is undertaken. (Ref : Annals of the Royal College Sergeants of England trainees presentation recommended by the college page 214- Department of vascular surgery and microbiology. WORCESTERSHIRE Royal Hospital U.K) which is essential to inform surgeons of their true rate in surgical site infections. To reduce the wound sepsis rate (as post operative wound closure (primary closure) during contaminated operations has been associated with nearly 40% wound sepsis rate based on Knightons wound severity index. (Knighton et al) and post surgical mortality rate, proper deduction and timely reporting of presence of ESBL producing GNB in post operative wounds is very essential. It is suggested that routine diagnosis of ESBL producing strains in post

operative wound infections has to be carried out inorder to avoid undesired effects of multi-drug resistant ESBL producing Klebsiella by strict control of antibiotic usage. The restricted use of antibiotics will lead to the withdrawal of selective pressure and resistant bacteria will no longer survive in such settings. These findings were the net result of this study. Further studies will be required to substantiate these findings in the field of microbiology.

SUMMARY

SUMMARY

- The results of this study suggest that the prevalence rate of ESBL producing gram negative bacilli in post operative wound infections was 37.5% which was within the range of 28-84% reported in India.
- The prevalence of ESBL identified in this study was found to co-exist with a high degree of co-resistance to non β lactum antibiotics. Therefore formulation of an appropriate Hospital Antibiotic Policy is necessary to control this infections.
- Over use of β lactams has imposed a selective pressure on pathogens to acquire resistance genes and mutate these confer a border range of activity. The following few points may m\be mentioned for the prevention and control of the spread of ESBLs.
- By having an effective and consistent approach to surveillance, prevention and control of the spread of ESBLs will be made possible.
- Screening of all targeted population at high risk and all high risk unit admissions, implementation of Hand washing programmes and basic cleaning of all surface levels. (Hand touch sites) were all necessary to control the spread.

- Out break activities in the form of patient screening, placing them in contact precautions user barrier precautions are all necessary.
- Our study highlights emergence of ESBL producing Klebsiella pneumoniae 48.01% isolates as one of the major multi drug resistant organisms.
- A multidisciplinary approach coordinated participation of microbiologists, clinicians, nursing personnel, hospital infection control team is necessary for the management of ESBL producing infections.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Protocol % 20 for % 20% ESBL % 20 – reporting – Protocol for ESBL Reporting.

2. An NCCLS Global Information Supplement – NCCLS – Screening and confirmatory tests for LSBLS – Jan. 2001. NCCLS Vol.21 – No.1.

3. MAQUET – ESBL

4. Indian Journal of Medical Microbiology – year 2004 – Vol. 22 – Issue 255-257 Shanmuganathan, C. Anandhakrishnan. A. Jaya Keerthi S.R, Kanungo. R, Bhattaharya.S, Badrinath – Learning from an outline. The essential points – Yun – Kuung, Hyunjoo Pai , Hoan – Jong Lee, others

5. Antimicrobial agents and chemotherapy; May 2002 p 1481-1491, Vol.46. No.5, Blood Stream Infection by ESBL – producing Escherichia coli and Klebsiella pneumoniae in children : epidemiology and clinical outcome :

6. Journal of clinical microbiological – Dec. 1999 P. 4167-4169 Vol. 37, No.12 – Molecular epidemiology of a club of cases due to Klebsiella pneumoniae producing SHV-5, ESBL in the Premature Intensive Care unit of a Hungarian Hospital : Dora S zabo, Zslot Filetoth, Juliana, Maria Nemedi, Carman Pena, Migull Pujol, Cammen Ardamy , Asumpta Ricent.

7. Anti-microbial Agents and Chemotherapy, January 1998 – Vol. 42 – No.1 – p 53-58 – Epidemiology. Successful control of a large outbreak due to Klebsiella pneumoniae productivity ESBLs.

8. Mitchell, Schwaber, Palli. M., Raney, J. Kamile Rasheed, Jamesh Biddle, Poctia William, John. E., Megrwan, Journal of Clinical Microbiology Jan. 2004 Vol. 42 : No.1 p 294-298. Utility of NCCLS guidelines for identifying ESBLs in Non Escherichia coli, S Klebsiella pneumoniae. Spp of Entrobacteriaceal.

9. Amita Jain, Indranil Roy, Mahenoha K. Gupta, Mala Kumar and S.K, Agarwal Journal if clinical microbiology 2003, 52; 421-425 : Prevalance of ESBL producing Gram negative bacteria in septicaemic neonates in a Tertiary Care Hospital Ding x 19 Shen, Douglas. J. Bledenbach.

10. Diagn Microbial Infect Dis – 1999. 34 – p 159-16, phenotypic and genotypic characterization of Chinese strains of Escherichia coli producing ESBLs.

11. U.Chaudhary, R. Aggarwal, Indian Journal of Medical Microbiology – 2004 2 22 (2) : 75 – 80 – ESBL – An emerging threat to clinical therapeutics.

12. Shakla. I, Tiwari. R, Agarwal. M. Dept. of Microbiology, J.N. Medical College, Aligarh University, Aligarh – Prevalence of ESBL producing Klebsiella pneumoniae in a Tertiary Care Hospital : year 2004, Vol. 22, Issue 2 : p87-91

13. A. Subha, S. Anandhan, Resistance to Third Generation cephalosporin among Klebsiella pneumoniae in Chennai. 2002-20(2) 92-95.

14. Ebbing Lautenbach, Jean Balduo Patel, Warmen. B Bieker, Paul H. Edel, Clinical Infection Diseases : 2001 – 32 : Neil O' Fishman 1162 – 71 – ESBL producing Escherichia coli and Klebsiella pneumoniae : Risk factors for infection factors and Impact of Resistance on outcomes.

15. A. Subha, S. Anandham B.s.V. Alavandi, Indian Journal Medi Res 113 May 2001 pp 181 – ESBL Production and Multi Drug Resistance in Kleb Species isolated from children under five with intestinal and extra intestinal infections.

16. Howard et al CID – 2003 : 36 (Suppl – 1) The Global Impact of Drug Resistance.

17. Michael. W. Carter, Karen J. Oakton, Maring Warner and David M. Livermore – Journal of clinical microbiology Nov. 2000, p 4228-4232 Vol. 38 – Detection of ESBLS in Klebsiella with the oxoid combination Disk method.

18. Reba Kanungo. Aswin. A. Anantha Kumar. A. Kumar and S. Badrinath – Indian Journal of Medical Microbiology, 2000 18(4); 160-165. Detection of ESBL producers – amond surgical wound infections and burns patients in JIPMER.

19. Nosocomial infection – Hospital today : Vol V No.4, April 2000. Beta lactamase mediated resistance in surgical pathogens.

20. Michael Gardem M.Sc; Md. CM. FRCPC. John Conlxy MD., FRCPC – Extended spectrum Beta lactam resistant Enterobacteriaceae; The Nosocmial pathogen jour? Where does the list end.

21. Vincent Jarlier. Mariee Helene. Nicolas. Genevieve Fournier Alain Phillippon – Reviews of infectious disease : Vol. 10 No.4 July, August 1988 – ESBLs conferring Transferable Resistance to newer Beta lactam Agents in Enterobacteriaceae : Hospital prevalence and susceptibility patterns.

22. D.L. Paterson – Clinical Microbiology and Infection 2000 : 6(9) 460 – 463 – Recommendation for treatment of severe infections caused by Enterobacteriaceae producing ESBLs.

23. Mary. S Farkosh. M.T. (ASCP) Infectous Diseases ESBL producing Gram negative bacilli : JHH.HEIC ESBL.

24. Jerestin B. Hansotia, Vandana Agarwal, A.A. Pattak and A.M. Saojii – Indian J. Med. Res 105 April 1997 pp.158-161. ESBL mediated resistance to third generation cephalosporins in Kleb pneumoniae in Nagpur – Central India.

25. A. Subha. S. Anandhan – ESBL mediated resistance to third generation cephalosporins among Klebsiella pneumoniae in Chennai. India Journal of micro Biology – 2002 20 (2) : 92-95.

26. Kenneth Thomson and Christine. C. Sandars – Anti Microbial Agents and chemotherapy Sep. 1992 p.1877 – 1882 Vol : 36 no.9 Detection of ESBLs in members of the family Enterobacteriaceae : comparision of the Double Disk and Three Dimensional Tests.

27. P.C. Rishi – Hospital Today Vol : V. No.7, July 2000 – Combination of B lactam antibiotics with Beta lactamase inhibitors.

28. C. Rodrigues, P. Joshi, S.H. Jani, M. Alphonse, R. Radhakrishnan, A. Mehta – Indian Journal of Medical Microbiology 2004 22(4) : 247 – 250 – Detection of B lactamases in Nosocomial Gram Negative Clinical Isolates.

29. C. Shanmugnathan, A. Ananthakrishnan, R. Kanungo, S.R. Jaya Keerthi, A. Kumar - Indian Journal of Medical Microbiology 2004 22(4) 255-257 Learning from an outbreak : ESBL : The Essential points.

30. PACTRICIA –A-BRADFORD – Clinical Microbiology Reviews – Oct. 2001 p 933-951 – Vol : 14 No. 4. Exteded Spectrum Beta lactamases in the 21st Century – characterization Epidimiology and Detection of This Important Resistance Threat.

31. Indian Journal of Medical Microbiology – Bhattarcharya. S 2006 Vol : 24 Issue I p 20-24 ESBL – from petridish to the patient.

32. Priya Datta, Archana Thakkur – Jpn.J.Infect Dis.57, 146-149 2004 prevalence of clinical strains resistant to various beta lactams in a Terittery Care Hospital in India.

33. FRED TENOVER, M. Jasmie Mohammed, Timothy. S Gorton and Zygmunt F.Dembek. Journal of Clinical Microbiology Dec. 1999 Vol : 37 No.12 p4065 – 4070 Detection and reporting o f organisms producing extended spectrum beta lactamases : survey of laboratories in Connecticut –

34. SCIEH weekly report – NHS – Supplement to SCIEH weekly report- 14 Dec. 2004 Vol : 38 No. 2004/50 - pages 1-8 extended spectrum Beta lactamases – are we prepared to face the threat.

35. Laboratry detection and reporting of Bacteria with ESBLs – NHS – National Standard Method – Guidance Notes – QSO P51 – National Public Health Service for Wales – p 1-14.

36. C. Friedman, C. Callery, A. Jenanes, P. Piaskowski, L. Scott and members of the Best Practicies – expert panel and reviewer – International infection control council – Best infection Control Practices for patients with ESBL Enterobacteriaceae.

37. CMC Winter Symposium III Abstract CMC Vellore Dec. 16th, 17th 2004 p. 32-33.

38. Beta-lactamases and detection of beta-lactam resistance in Enterobacter spp JD Pitout, ES Moland, CC Sanders, KS Thomson and SR Fitzsimmons.

39. AmpC Disk Test for Detection of Plasmid-Mediated AmpC ß-Lactamases in Enterobacteriaceae Lacking Chromosomal AmpC ß-Lactamases Jennifer A. Black, Ellen Smith Moland, and Kenneth S. Thomson*.

40. Virulence factors of Escherichia coli isolates that produce CTX-M-type extended-spectrum beta-lactamases. Pitout JD, Laupland KB, Church DL, Menard ML, Johnson JR. Year : 1999 | Volume : 17 | Issue : 4 | Page : 160-165.

41. Detection of extended spectrum BETA-LACTAMASE produces among surgical wound infections and burns patients in JIPER Ananthakrishnan AN, Kanungo R Journal of Clinical Microbiology, 04 1996, 908-911, Vol 34, No. 4.

42. Detection of extended-spectrum beta-lactamases in clinical isolates of Klebsiella pneumoniae and Escherichia coli GA Jacoby and P Han Lahey Hitchcock Clinic, Burlington, Massachusetts 01805, USA.

43. Antimicrobial Agents and Chemotherapy, December 2002, p. 3739-3743, Vol. 46, No. 12 0066-4804/02/$04.00+0 DOI: 10.1128/AAC.46.12.3739-3743.2002 Distribution of Extended-Spectrum ß-Lactamases in Clinical Isolates of Enterobacteriaceae in Vietnam Van Cao,1,2 Thierry Lambert,1,3* Duong Quynh Nhu,2 Huynh Kim Loan,2 Nguyen Kim Hoang,2 Guillaume Arlet,4 and Patrice Courvalin1 Unité des Agents Antibactériens, Institut Pasteur, 75724 Paris Cedex 15,1 Centre d'Etude Pharmaceutiques, ChÂtenay-Malabry,3 Service de Bactériologie, Hôpital Tenon, U.F.R Saint-Antoine 75970 Paris Cedex 20, France,4 Institut Pasteur d'Ho Chi Minh Ville, Ho Chi Minh City, Vietnam2

44. J Commun Dis. 2004 Mar;36(1):17-26. Related Articles, Links Antimicrobial susceptibility pattern and prevalence of extended spectrum beta-lactamase (ESBLs) producing strains of Klebsiella pneumoniae from a major hospital in New Delhi. Grover SS, Sharma M, Pasha ST, Singh G, Lal S. National Institute of Commnnnunicable Diseases, 22-Shamnath Marg, Delhi-10054.

45. J Pak Med Assoc. 2005 Oct;55(10):436-9. Related Articles, Links Frequency and sensitivity pattern of Extended Spectrum beta Lactamase producing isolates in a tertiary care hospital laboratory of Pakistan. Jabeen K, Zafar A, Hasan R. Department of Pathology, The Aga Khan University Hospital, Karachi, Pakistan.

46. Int J Antimicrob Agents. 2000 Mar;14(2):137-42 Related Articles, Links CTX-M-type beta-lactamases: an emerging group of extended-spectrum enzymes. Tzouvelekis LS, Tzelepi E, Tassios PT, Legakis NJ. Department of Microbiology, Medical School, National University of Athens, M. Asias 75, Athens, Greece.

47. SPECIAL ARTICLE Year : 2006 | Volume : 24 | Issue : 1 | Page : 20-24 ESBL- From petri dish to the patient Bhattacharya S Health Protection Agency, Heart of England NHS Trust, Bordesley Green East, Birmingham, B9 5SS, India. Journal of Clinical Microbiology, August 2002, p. 2755-2759, Vol. 40, No. 80095-1137/02/$04.00+0 DOI: 10.1128/ JCM.40.8.2755 -2759.2002 Evaluation of a New Etest for Detecting Metallo-ß-Lactamases in Routine Clinical Testing Timothy R. Walsh,1* Anne Bolmström,2 Anette Qwärnström,2 and Ana Gales3 University of Bristol, Bristol, United Kingdom,1 AB BIODISK, Solna, Sweden,2 University of Iowa, Iowa City, Iowa3 .

48. Antimicrobial Agents and Chemotherapy, August 2003, p. 2615-2618, Vol. 47, No. 8 0066-4804/03/$08.00+0 DOI: 10.1128/AAC.47.8.2615-2618.2003 In Vitro Activities of Novel Oxapenems, Alone and in Combination with Ceftazidime, against Gram-Positive and Gram-Negative Organisms Conor E. Jamieson,1 Peter A. Lambert,1* and Iain N. Simpson2 Life and Health Sciences, Aston University, Birmingham,1 Micron Research, Cambridge, United Kingdom2.

49. Journal of Clinical Microbiology, August 2005, p. 4163-4167, Vol. 43, No. 80095-1137/05/$08.00+0 doi:10.1128/ JCM.43.8.4163-4167.2005 Inhibitor-Based Methods for Detection of Plasmid-

Mediated AmpC ß-Lactamases in Klebsiella spp., Escherichia coli, and Proteus mirabilis Philip E. Coudron*.

50. Pathology and Laboratory Medicine Service/113, McGuire Veterans Affairs, Medical Center, Richmond, Virginia 23249-0001 Antimicrobial Agents and Chemotherapy, December 2002, p. 3837-3842, Vol. 46, No. 12 0066-4804/02/$04.00+0 DOI:10.1128/AAC.46.12.3837-3842.2002 Occurrence of Newer ß-Lactamases in Klebsiella pneumoniae Isolates from 24 U.S. Hospitals Ellen Smith Moland, Jennifer A. Black, Jason Ourada, Mark D. Reisbig, Nancy D. Hanson, and Kenneth S. Thomson* Center for Research in Antiinfectives and Biotechnology, Department of Medical Microbiology and Immunology, Creighton University School of Medicine, Omaha, Nebraska 68178.

51. Journal of Clinical Microbiology, October 2005, p. 5058-5064, Vol. 43, No. 100095-1137/ 05/$08.00+0 doi:10.1128/JCM.43.10.5058-5064.2005.

52. SHV-Type Extended-Spectrum Beta-Lactamase Production Is Associated with Reduced Cefepime Susceptibility in Enterobacter cloacae Dóra Szabó,1,2 Robert A. Bonomo,3 Fernanda Silveira,1 A. William Pasculle,4 Carla Baxter,4 Peter K. Linden,5 Andrea M. Hujer,3 Kristine M. Hujer,3 Kathleen Deeley,1 and David L. Paterson1* Division of Infectious Diseases, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania 15213,1 Institute of Medical Microbiology, Semmelweis University, Budapest, Hungary,2 Research Service, Louis Stokes Cleveland Veterans Affairs Medical Center, Cleveland, Ohio 44106,3 Clinical Microbiology Laboratory, University of Pittsburgh Medical Center,

Pittsburgh, Pennsylvania 15213,4 Department of Critical Care Medicine, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania 152135.

53. New Microbiol. 2005 Jul;28(3):231-6. An outbreak of SHV-5 producing Klebsiella pneumoniae in a neonatal intensive care unit; meropenem failed to avoid fecal colonization. Gundes S, Arisoy AE, Kolayli F, Karaali E, Turker G, Sanic A, Arisoy ES, Vahaboglu H. Infeksiyon Hastaliklari & Klinik Mikrobiyoloji AD, Kocaeli Universitesi, Kocaeli, Turkey.

54. Mackie and McCartney Practical Medical Biology 14th Edition.

55. Antimicrobial Agents and Chemotherapy, 11 1996, 2488-2493, Vol 40, No. 11 A TEM-derived extended-spectrum beta-lactamase in Pseudomonas aeruginosa P Mugnier, P Dubrous, I Casin, G Arlet and E Collatz Laboratoire de Recherche Moleculaire sur les Antibiotiques, Universite Paris VI, France.

56. Journal of Clinical Microbiology, February 2000, p. 570-574, Vol. 38, No. 2 0095-1137/00/$04.00+0 Ability of the VITEK 2 Advanced Expert System To Identify-Lactam Phenotypes in Isolates of Enterobacteriaceae and Pseudomonas aeruginosa.

57. Christine C. Sanders,1 Michel Peyret,2 Ellen Smith Moland,1 Carole Shubert,2 Kenneth S. Thomson,1,* Jean-Marc Boeufgras,3 and W. Eugene Sanders Jr.1 Center for Research in Anti-Infectives and Biotechnology, Department of Medical Microbiology and Immunology, Creighton University School of Medicine, Omaha, Nebraska 681781; bioMérieux Inc., Hazelwood, Missouri 630422; and bioMérieux, LaBalme-Les-Grottes, France3.

58. Journal of Clinical Microbiology, July 2005, p. 3110-3113, Vol. 43, No. 70095-1137/05/$08.00+0 doi:10.1128/JCM.43.7.3110-3113.2005 AmpC Disk Test for Detection of Plasmid-Mediated AmpC ß-Lactamases in Enterobacteriaceae Lacking Chromosomal AmpC ß-Lactamases.

59. Jennifer A. Black, Ellen Smith Moland, and Kenneth S. Thomson* Center for Research in Antiinfectives and Biotechnology, Department of Medical Microbiology and Immunology, Creighton University School of Medicine, Omaha, Nebraska.

60. Antimicrobial Agents and Chemotherapy, May 1999, p. 1281-1284, Vol. 43, No. 50066-4804/99/$04.00+0 Service de Bactériologie-Virologie, Hôpital de Bicêtre, Faculté de Médecine Paris-Sud, 94275 Le Kremlin-Bicêtre,1 Service de Bactériologie-Virologie, Hôpital Antoine Béclère, Faculté de Médecine Paris-Sud, 92141 Clamart Cedex,2 and Service de Microbiologie, Hôpital Raymond Poincaré, Faculté de Médecine Paris-Ouest, 92380 Garches,3 Assistance Publique/Hôpitaux de Paris, France.

61. Journal of Antimicrobial Chemotherapy (1996) 38, 409-424 © 1996 The British Society for Antimicrobial Chemotherapy Antibiotic resistance and production of extended-spectrum ß-Mactamases amongst Klebsiella spp. from intensive care units in Europe D. M. Livermore and M. Yuan Department of Medical Microbiology, The London Hospital Medical College Turner Street, London E1 2AD, UK.

62. J Hosp Infect. 1997 Jan;35(1):9-16. Risk factors for faecal carriage of Klebsiella pneumoniae producing extended spectrum beta-lactamase (ESBL-KP) in the intensive care unit. Pena C, Pujol M,

Ricart A, Ardanuy C, Ayats J, Linares J, Garrigosa F, Ariza J, Gudiol F. Infectious Disease Service, Hospital de Bellvitge, Universidad de Barcelona, Spain.

63. Antimicrobial Agents and Chemotherapy, 01 1997, 35-39, Vol 41, No. 1 Beta-lactamases and detection of beta-lactam resistance in Enterobacter spp JD Pitout, ES Moland, CC Sanders, KS Thomson and SR Fitzsimmons Department of Medical Microbiology and Immunology, Creighton University School of Medicine, Omaha, Nebraska 68178, USA.

64. Clinical Microbiology Reviews, Oct 1995, 557-584, Vol 8, No. 4 beta-Lactamases in laboratory and clinical resistance DM Livermore Department of Medical Microbiology, London Hospital Medical College, United Kingdom.

65. Antimicrob Agents Chemother. 2005 Nov;49(11):4667-70. Virulence factors of Escherichia coli isolates that produce CTX-M-type extended-spectrum beta-lactamases. Pitout JD, Laupland KB, Church DL, Menard ML, Johnson JR. Department of Pathology and Laboratory Medicine, University of Calgary, Alberta, Canada. johann.pitout@cls.ab.ca.

66. Antimicrob. Agents Chemother., 11 1997, 2374-2382, Vol 41, No. 11 Characterization of a new TEM-derived beta-lactamase produced in a Serratia marcescens strain M Perilli, A Felici, N Franceschini, A De Santis, L Pagani, F Luzzaro, A Oratore, GM Rossolini, JR Knox and G Amicosante Dipartimento di Scienze e Tecnologie Biomediche, Cattedra di Chimica Biologica, Facolta di Medicina e Chirurgia, Universita degli Studi dell'Aquila, L'Aquila, Italy.

67. Journal of Antimicrobial Chemotherapy (1996) 38, 183-191 © 1996 The British Society for Antimicrobial Chemotherapy Antimicrobial Agents and Chemotherapy, July 2000, p. 1970-1973, Vol. 44, No. 70066-4804/00/$04.00+0 Cloning and Sequence of the Gene Encoding a Novel Cefotaxime Hydrolyzing -Lactamase (CTX-M-9) from Escherichia coli in Spain Montserrat Sabaté,1 Raül Tarragó,2 Ferran Navarro,1,* Elisenda Miró,1 Clara Vergés,1 Jordi Barbé,2 and Guillermo Prats1 Departament de Microbiologia, Hospital de la Santa Creu i Sant Pau, Universitat Autònoma 08025 Barcelona,1 and Departament de Genètica i Microbiologia, Universitat Autònoma de Barcelona, 08193 Bellaterra,2 Spain.

68. Antimicrobial Agents and Chemotherapy, May 1998, p. 1181-1186, Vol. 42, No. 5 0066-4804/98/$04.00+0 Cloning and Sequencing of the Gene Encoding Toho-2, a Class A -Lactamase Preferentially Inhibited by Tazobactam Ling Ma,1 Yoshikazu Ishii,1,* Masaji Ishiguro,2 Hiroshi Matsuzawa,3 and Keizo Yamaguchi1 Department of Microbiology, Toho University School of Medicine, 5-21-16 Omori-nishi, Ota-ku, Tokyo 143-8540,1 Suntory Institute for Bioorganic Research, 1-1 Wakayamadai, Shimamoto, Osaka 618-8503,2 and Department of Biotechnology, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657,3 Japan.

69. Journal of Clinical Microbiology, 08 1997, 2061-2067, Vol 35, No. 8 Detection and clinical significance of extended-spectrum beta-lactamases in a tertiary-care medical center CL Emery and LA Weymouth Department of Pathology, Medical College of

Virginia, Virginia Commonwealth University, Richmond 23298, USA.

70. Journal of Clinical Microbiology, February 2000, p. 542-546, Vol. 38, No. 20095-1137/00/$04.00+0 Detection of Extended-Spectrum-Lactamases in Clinical Isolates of Enterobacter cloacae and Enterobacter aerogenes Eva Tzelepi,1 Panagiota Giakkoupi,1 Danai Sofianou,2 Veneta Loukova,1 Anastassia Kemeroglou,2 and Athanassios Tsakris3,* Department of Bacteriology, Hellenic Pasteur Institute, 115 21 Athens,1 Department of Microbiology, Hippokration General Hospital, 54642 Thessaloniki,2 and Department of Microbiology, Medical School, Aristotle University of Thessaloniki, 54006 Thessaloniki, 3 Greece.

71. Journal of Clinical Microbiology, November 2000, p. 4228-4232, Vol. 38, No. 110095-1137/00/$04.00+0 Journal of Clinical Microbiology, 08 1996, 1880-1884, Vol 34, No. 8 Detection of extended-spectrum beta-lactamase (ESBL)-producing strains by the Etest ESBL screen MG Cormican, SA Marshall and RN Jones Department of Pathology, University of Iowa College of Medicine, Iowa City 52242, USA.

72. Journal of Clinical Microbiology, 04 1996, 908-911, Vol 34, No. 4 Journal of Clinical Microbiology, 12 1996, 2997-3001, Vol 34, No. 12 CC Sanders, AL Barry, JA Washington, C Shubert, ES Moland, MM Traczewski, C Knapp and R Mulder Center for Research in Anti-Infectives and Biotechnology, Creighton University, School of Medicine, Omaha, Nebraska 68178, USA.

73. Antimicrobial Agents and Chemotherapy, December 2002, p. 3739-3743, Vol. 46, No. 120066-4804/02/$04.00+0 DOI:

10.1128/AAC.46.12.3739-3743.2002 Distribution of Extended-Spectrum ß-Lactamases in Clinical Isolates of Enterobacteriaceae in Vietnam Van Cao,1,2 Thierry Lambert,1,3* Duong Quynh Nhu,2 Huynh Kim Loan,2 Nguyen Kim Hoang,2 Guillaume Arlet,4 and Patrice Courvalin1 Unité des Agents Antibactériens, Institut Pasteur, 75724 Paris Cedex 15,1 Centre d'Etude Pharmaceutiques, ChÂtenay-Malabry,3 Service de Bactériologie, Hôpital Tenon, U.F.R Saint-Antoine 75970 Paris Cedex 20, France,4 Institut Pasteur d'Ho Chi Minh Ville, Ho Chi Minh City, Vietnam2.

74. Antimicrobial Agents and Chemotherapy, Aug 1995, 1878-1880, Vol 39, No. 8 Effects of Asp-179 mutations in TEMpUC19 beta-lactamase on susceptibility to beta-lactams SB Vakulenko, M Toth, P Taibi, S Mobashery and SA Lerner Department of Medicine, Wayne State University, Detroit, Michigan 48202, USA.

75. Antimicrobial Agents and Chemotherapy, 04 1997, 838-840, Vol 41, No. 4 Emergence of an inhibitor-resistant beta-lactamase (SHV-10) derived from an SHV-5 variant EE Prinarakis, V Miriagou, E Tzelepi, M Gazouli and LS Tzouvelekis Department of Bacteriology, Hellenic Pasteur Institute, Athens, Greece.

76. J Infect. 1998 Nov;37(3):274-81. Molecular epidemiology as an effective tool in the surveillance of infections in the neonatal intensive care unit. Villari P, Iacuzio L, Torre I, Scarcella A. Institute of Hygiene and Preventive Medicine, University Federico II, Naples, Italy.

77. Antimicrob Agents Chemother. 1988 Feb;32(2):175-9. Detection of plasmid-mediated beta-lactamases with DNA probes. Huovinen S, Huovinen P, Jacoby GA. Massachusetts General Hospital, Boston 02114.

78. Antimicrob Agents Chemother. 1988 Aug;32(8):1243-6. Novel plasmid-mediated beta-lactamase from Escherichia coli that inactivates oxyimino-cephalosporins. Matsumoto Y, Ikeda F, Kamimura T, Yokota Y, Mine Y. Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan.

79. APMIS. 2000 Mar;108(3):237-40.Comparison of screening methods for detection of extended-spectrum beta-lactamases and their prevalence among Escherichia coli and Klebsiella species in Hong Kong. Ho PL, Tsang DN, Que TL, Ho M, Yuen KY. Department of Microbiology, Queen Mary Hospital, University of Hong Kong, Pokfulam, China. plho@hkucc.hku.hk.

80. J Commun Dis. 2004 Mar;36(1):17-26. Antimicrobial susceptibility pattern and prevalence of extended spectrum beta-lactamase (ESBLs) producing strains of Klebsiella pneumoniae from a major hospital in New Delhi. Grover SS, Sharma M, Pasha ST, Singh G, Lal S. National Institute of Commnnnunicable Diseases, 22-Shamnath Marg, Delhi-10054.

81. J Pak Med Assoc. 2005 Oct;55(10):436-9. Frequency and sensitivity pattern of Extended Spectrum beta Lactamase producing isolates in a tertiary care hospital laboratory of Pakistan. Jabeen K, Zafar A, Hasan R. Department of Pathology, The Aga Khan University Hospital, Karachi, Pakistan.

82. Int J Antimicrob Agents. 2000 Mar;14(2):137-42. CTX-M-type beta-lactamases: an emerging group of extended-spectrum enzymes. Tzouvelekis LS, Tzelepi E, Tassios PT, Legakis NJ. Department of Microbiology, Medical School, National University of Athens, M. Asias 75, Athens, Greece.

83. Antimicrob Agents Chemother. 1990 Apr;34(4):581-6. Activity of sulbactam in combination with ceftriaxone in vitro and in experimental endocarditis caused by Escherichia coli producing SHV-2-like beta-lactamase. Fantin B, Pangon B, Potel G, Caron F, Vallee E, Vallois JM, Mohler J, Bure A, Philippon A, Carbon C. Institut National de la Sante et de la Recherche Medicale U:13, Hopital Claude Bernard, Paris, France.

84. Journal of Antimicrobial Chemotherapy, Vol 41, 527-539, Copyright © 1998 by The British Society for Antimicrobial Chemotherapy.

85. Epidemiological typing of klebsiellae with extended-spectrum beta- lactamases from European intensive care units M Yuan, H Aucken, LM Hall, TL Pitt and DM Livermore Antibiotic Group, Department of Medical Microbiology, St Bartholomew's and the Royal London School of Medicine and Dentistry, Whitechapel Site, London, UK.

86. Antimicrobial Agents and Chemotherapy, January 1998, p. 53-58, Vol. 42, No. 10066-4804/98/$04.00+0 Epidemiology and Successful Control of a Large Outbreak Due to Klebsiella pneumoniae Producing ExtendedSpectrum - Lactamases Carmen Peña,1,* Miquel Pujol,1 Carmen Ardanuy,2 Asumpta Ricart,3 Roman Pallares,1 Josefina Liñares,2 Javier Ariza,1 and Francisco

Gudiol1 Infectious Disease Service,1 Microbiology Service,2 and Intensive Care Unit Service,3 Hospital de Bellvitge, Universidad de Barcelona, Barcelona, Spain.

87. Rev Infect Dis. 1988 Jul-Aug;10(4):867-78. Extended broad-spectrum beta-lactamases conferring transferable resistance to newer beta-lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. Jarlier V, Nicolas MH, Fournier G, Philippon A. Service de Bacterologie, Faculte de Medecine, Pitie-Salpetriere, France.

88. Journal of Clinical Microbiology, August 2002, p. 2755-2759, Vol. 40, No. 80095-1137/02/$04.00+0 DOI: 10.1128/ JCM.40.8.2755-2759.2002 Evaluation of a New Etest for Detecting Metallo-ß-Lactamases in Routine Clinical Testing Timothy R. Walsh,1* Anne Bolmström,2 Anette Qwärnström,2 and Ana Gales3 University of Bristol, Bristol, United Kingdom,1 AB BIODISK, Solna, Sweden,2 University of Iowa, Iowa City, Iowa3

89. Journal of Clinical Microbiology, October 1998, p. 3105-3110, Vol. 36, No. 100095-1137 /98/$04.00+0 Extended-Spectrum -Lactamase-Producing Klebsiella pneumoniae Strains Causing Nosocomial Outbreaks of Infection in the United Kingdom Kevin Shannon,1,* Paul Stapleton,1 Xiaoqin Xiang,1, Alan Johnson,2 Hamish Beattie,2 Fatima El Bakri,2, Barry Cookson,2 and Gary French1 Department of Microbiology, United Medical and Dental Schools, St. Thomas's Campus, London SE1 7EH,1 and Laboratory of Hospital Infection, PHLS Central Public Health Laboratory, London NW9 5HT,2 United Kingdom.

90. Antimicrobial Agents and Chemotherapy, November 2000, p. 3239-3240, Vol. 44, No. 110066-4804/00/$04.00+0 LETTERS TO THE EDITOR First Isolation of a CTX-M-3-Producing Enterobacter cloacae in France.

91. Antimicrob Agents Chemother. 1990 Nov;34(11):2193-9. Outbreak of ceftazidime resistance caused by extended-spectrum beta-lactamases at a Massachusetts chronic-care facility. Rice LB, Willey SH, Papanicolaou GA, Medeiros AA, Eliopoulos GM, Moellering RC Jr, Jacoby GA. New England Deaconess Hospital, Boston, Massachusetts 02115.

92. Antimicrobial Agents and Chemotherapy, August 2003, p. 2615-2618, Vol. 47, No. 80066-4804/03/$08.00+0 DOI: 10.1128/AAC.47.8.2615-2618.2003 In Vitro Activities of Novel Oxapenems, Alone and in Combination with Ceftazidime, against Gram-Positive and Gram-Negative Organisms Conor E. Jamieson,1 Peter A. Lambert,1* and Iain N. Simpson2 Life and Health Sciences, Aston University, Birmingham,1 Micron Research, Cambridge, United Kingdom2.

93. Journal of Clinical Microbiology, August 2005, p. 4163-4167, Vol. 43, No. 80095-1137/05/$08.00+0 doi:10.1128 /JCM.43.8.4163-4167.2005 Inhibitor-Based Methods for Detection of Plasmid-Mediated AmpC ß-Lactamases in Klebsiella spp., Escherichia coli, and Proteus mirabilis Philip E. Coudron* Pathology and Laboratory Medicine Service/113, McGuire Veterans Affairs, Medical Center, Richmond, Virginia 23249-0001.

94. Journal of Antimicrobial Chemotherapy (1999) 43, 447-458 © 1999 The British Society for Antimicrobial Chemotherapy Review Inhibitor-resistant TEM ß-lactamases: phenotypic, genetic and biochemical characteristics E. B. Chaïbia, D. Sirotb, G. Paulc and R. Labiaa,* a UMR 175, CNRS-MNHN, 6 Rue de l'Université, 29000 Quimper b Laboratoire de Bactériologie, Faculté de Médecine, 28 Place Henri-Dunant, 63001 Clermont-Ferrand Cedex c CHU Cochin, Laboratoire de Bactériologie, 75014 Paris, France.

95. Antimicrobial Agents and Chemotherapy, March 2000, p. 760-762, Vol. 44, No. 30066-4804 /00/$04.00+0 Capnocytophaga ochracea: Characterization of a Plasmid-Encoded Extended-Spectrum TEM-17 β-Lactamase in the Phylum Flavobacter-Bacteroides Agnes Rosenau,1,* Blandine Cattier,1 Nathalie Gousset,1 Patrick Harriau,1 Alain Philippon,2 and Roland Quentin1 Département de Microbiologie Médicale et Moléculaire, Center Hospitalier Universitaire Bretonneau, 37044 Tours cedex,1 and Service de Bactériologie, Hôpital Cochin (Université Paris V), 75679 Paris cedex 14,2 France.

96. J Hosp Infect. 1998 Aug;39(4):291-300. Comment in: J Hosp Infect. 1999 Jan;41(1):71-2. A hospital outbreak of extended-spectrum beta-lactamase-producing Klebsiella pneumoniae investigated by RAPD typing and analysis of the genetics and mechanisms of resistance. Shannon K, Fung K, Stapleton P, Anthony R, Power E, French G. Department of Microbiology, UMDS, St Thomas's Hospital, London, UK. Vol. 280 No. 14, October 14, 1998 Original Contribution Class Restriction of Cephalosporin Use to Control Total Cephalosporin Resistance in Nosocomial Klebsiella James J. Rahal, MD; Carl Urban, PhD;

David Horn, MD; Katherine Freeman, DrPH; Sorana Segal-Maurer, MD; James Maurer, MD; Noriel Mariano, BS; Sheila Marks, RN; Janice M. Burns, RN; Dana Dominick, RPh, MS; Mimi Lim, RN, MPA JAMA. 1998;280:1233-1237.

97. Antimicrobial Agents and Chemotherapy, August 1998, p. 2074-2083, Vol. 42, No. 80066-4804/98/$04.00+0 Molecular Characterization of OXA-20, a Novel Class D - Lactamase, and Its Integron from Pseudomonas aeruginosa Thierry Naas,1,* Wladimir Sougakoff,2 Anne Casetta,3 and Patrice Nordmann1,3.

98. Eur J Clin Microbiol Infect Dis. 1996 May;15(5):398-402. Detection of genes coding for extended-spectrum SHV beta-lactamases in clinical isolates by a molecular genetic method, and comparison with the E test. Nuesch-Inderbinen MT, Hachler H, Kayser FH. Department of Medical Microbiology, University of Zurich, Switzerland.

99. Antimicrobial Agents and Chemotherapy, May 1995, 1199-1200, Vol 39, No. 5 New variant of TEM-10 beta-lactamase gene produced by a clinical isolate of proteus mirabilis T Palzkill, KS Thomson, CC Sanders, ES Moland, W Huang and TW Milligan Department of Microbiology and Immunology, Baylor College of Medicine, Houston, TX 77030, USA.

100. Clin Infect Dis. 1998 Aug;27 Suppl 1:S100-6. Trends in beta-lactam resistance among Enterobacteriaceae. Nordmann P. Hopitaux de Bicetre et Antoine Becleere, Hopitaux de Paris, Faculte Medecine Paris-Sud, France.

101. Antimicrobial Agents and Chemotherapy, December 1998, p. 3113-3116, Vol. 42, No. 120066-4804/98/$04.00+0 Novel OXA-10-Derived Extended-Spectrum -Lactamases Selected In Vivo or In Vitro P. Mugnier,1, I. Casin,1,2 A. T. Bouthors,1 and E. Collatz1,* Laboratoire de Recherche Moléculaire sur les Antibiotiques, UFR Broussais-Hôtel Dieu and UFR Pitié-Salpêtrière, Université Paris VI,1 and Laboratoire de Bactériologie, Hôpital Saint-Louis, Université Paris VII,2 Paris, France.

102. Antimicrobial Agents and Chemotherapy, December 2002, p. 3837-3842, Vol. 46, No. 12 0066-4804/02/$04.00+0 DOI: 10.1128/AAC.46.12.3837-3842.2002 Occurrence of Newer ß-Lactamases in Klebsiella pneumoniae Isolates from 24 U.S. Hospitals Ellen Smith Moland, Jennifer A. Black, Jason Ourada, Mark D. Reisbig, Nancy D. Hanson, and Kenneth S. Thomson* Center for Research in Antiinfectives and Biotechnology, Department of Medical Microbiology and Immunology, Creighton University School of Medicine, Omaha, Nebraska 68178.

103. Antimicrob Agents Chemother. 1988 Mar;32(3):397-9. Oligonucleotide probes (TEM-1, OXA-1) versus isoelectric focusing in beta-lactamase characterization of 114 resistant strains. Ouellette M, Paul GC, Philippon AM, Roy PH. Department de Biochimie, Faculte des Sciences et de Genie, Universite Laval, Ste-Foy, Quebec, Canada.

104. Antimicrobial Agents and Chemotherapy, 08 1995, 1881-1884, Vol 39, No. 8 OXA-14, another extended-spectrum variant of OXA-10 (PSE-2) beta- lactamase from Pseudomonas aeruginosa F Danel, LM Hall, D Gur and DM Livermore Department of Medical

Microbiology, London Hospital Medical College, United Kingdom.

105. Antimicrobial Agents and Chemotherapy, 04 1997, 785-790, Vol 41, No. 4 OXA-15, an extended-spectrum variant of OXA-2 beta-lactamase, isolated from a Pseudomonas aeruginosa strain F Danel, LM Hall, D Gur and DM Livermore Department of Medical Microbiology, St. Bartholomew's and the Royal London School of Medicine and Dentistry, London, United Kingdom. F.H.Danel@mds.qmw.ac.uk.

106. Antimicrobial Agents and Chemotherapy, December 1998, p. 3117-3122, Vol. 42, No. 120066-4804/98/$04.00+0 OXA-16, a Further Extended-Spectrum Variant of OXA-10 -Lactamase, from Two Pseudomonas aeruginosa Isolates Franck Danel,1,* Lucinda M. C. Hall,1 Deniz Gur,2 and David M. Livermore1, Antibiotic Group, Department of Medical Microbiology, St. Bartholomew's and the Royal London School of Medicine and Dentistry, London, E1 2AD, United Kingdom,1 and Section of Infectious Diseases, Department of Internal Medicine, Hacettepe University School of Medicine, 06100 Ankara, Turkey2.

107. Antimicrobial Agents and Chemotherapy, June 1999, p. 1362-1366, Vol. 43, No. 6 0066-4804/99/$04.00+0 OXA-17, a Further Extended-Spectrum Variant of OXA-10 -Lactamase, Isolated from Pseudomonas aeruginosa Franck Danel,1,* Lucinda M. C. Hall,1 Brigid Duke,1 Deniz Gur,2 and David M. Livermore1, Antibiotic Group, Department of Medical Microbiology, St. Bartholomew's and the Royal London School of Medicine and Dentistry, London, E1 2AD, United Kingdom,1 and

Section of Infectious Diseases, Department of Internal Medicine, Hacettepe University School of Medicine, 06100 Ankara, Turkey2.

108. Antimicrob. Agents Chemother., 10 1997, 2188-2195, Vol 41, No. 10 OXA-18, a class D clavulanic acid-inhibited extended-spectrum beta- lactamase from Pseudomonas aeruginosa LN Philippon, T Naas, AT Bouthors, V Barakett and P Nordmann Service de Bacteriologie-Virologie, Hopital Antoine Beclere, Faculte de Medecine Paris-Sud, Clamart, France.

109. Antimicrobial Agents and Chemotherapy, February 2001, p. 447-453, Vol. 45, No. 20066-4804/01/$04.00+0 DOI: 10.1128/AAC.45.2.447-453.2001OXA-28, an Extended-Spectrum Variant of OXA-10 -Lactamase from Pseudomonas aeruginosa and Its Plasmid- and Integron-Located Gene Laurent Poirel, Delphine Girlich, Thierry Naas, and Patrice Nordmann*

110. Clinical Infectious Diseases 1999;29:1419-1422© 1999 by the Infectious Diseases Society of America. All rights reserved. 1058-4838/1999/2906-0011$03.00. Editorial Response: Extended-Spectrum β-Lactamases: A Call for Improved Detection and Control David L. Paterson and Victor L. Yu Infectious Disease Section, Veterans Affairs Medical Center, Pittsburgh, Pennsylvania.

111. Journal of Clinical Microbiology, March 1998, p. 827-829, Vol. 36, No. 30095-1137/98/$04.00+0 Practical Approach for Detection and Identification of OXA-10-Derived Ceftazidime-Hydrolyzing Extended-Spectrum Error! Unknown switch argument.-Lactamases Haluk Vahaboglu,1,* Recep Ozturk,2 Huriye Akbal,2 Suat

Saribas,2 Ozlem Tansel,1 and Figen CoError! Unknown switch argument.kunkan1

112. Journal of Antimicrobial Chemotherapy (1990) 26, 635-648 © 1990 The British Society for Antimicrobial Chemotherapy research-article. Prevalence of a transferable SHV-5 type ß-lactamase in clinical isolates of Klebsiella pneumoniae and Escherichia coli in Greece A. C. Vatopoulosa, A. Philipponb, L. S. Tzouvelekisc, Z. Komninoua and N. J. Legakisd.

113. Antimicrob Agents Chemother. 1991 Jan;35(1):164-9. Properties of plasmids responsible for production of extended-spectrum beta-lactamases. Jacoby GA, Sutton L. Massachusetts General Hospital, Boston 02114.

114. Antimicrobial Agents and Chemotherapy, July 2000, p. 1860-1864, Vol. 44, No. 70066-4804/00/$04.00+0 Rapid Discriminatory Detection of Genes Coding for SHV -Lactamases by Ligase Chain Reaction Jungmin Kim1,* and Hoan-Jong Lee2 Department of Microbiology, College of Medicine, Dankook University, Cheonan,1 and Department of Pediatrics, Seoul National University College of Medicine, Seoul,2 Korea.

115. Clin Infect Dis. 1996 Jul;23(1):118-24. Ceftazidime-resistant Klebsiella pneumoniae isolates recovered at the Cleveland Department of Veterans Affairs Medical Center. Rice LB, Eckstein EC, DeVente J, Shlaes DM. Research Service, Department of Veterans Affairs Medical Center, Cleveland, Ohio 44106, USA.

116. FEMS Microbiology Letters Volume 92, Issue 1 , 1 April 1992, Pages 89-94 doi:10.1016/0378-1097(92)90547-2 Does high level

production of SHV-type penicillinase confer resistance to ceftazidime in enterobacteriaceae? Petita, H. Ben Yaghlane-Bouslamab, L. Sofera and R. Labiaa.

117. FEMS Microbiol Lett. 2000 Mar 1;184(1):85-9. Characterisation of extended-spectrum beta-lactamases of the SHV family using a combination of PCR-single strand conformational polymorphism (PCR-SSCP) and PCR-restriction fragment length polymorphism (PCR-RFLP). Chanawong A, M'Zali FH, Heritage J, Lulitanond A, Hawkey PM. Department of Microbiology, University of Leeds, Leeds, UK.

118. Curr Pharm Des. 1999 Nov;5(11):847-64. SHV-type beta-lactamases. Tzouvelekis LS, Bonomo RA. Laboratory of Antimicrobial Agents, Department of Microbiology, Medical School, National University of Athens, Greece.

119. Journal of Clinical Microbiology, October 2005, p. 5058-5064, Vol. 43, No. 10 0095-1137/05/$08.00+0 doi:10.1128/JCM.43.10.5058-5064.2005 SHV-Type Extended-Spectrum Beta-Lactamase Production Is Associated with Reduced Cefepime Susceptibility in Enterobacter cloacae Dóra Szabó,1,2 Robert A. Bonomo,3 Fernanda Silveira,1 A. William Pasculle,4 Carla Baxter,4 Peter K. Linden,5 Andrea M. Hujer,3 Kristine M. Hujer,3 Kathleen Deeley,1 and David L. Paterson1*

120. Occurrence of Extended-Spectrum Betalactamases (ESBL) in Dutch Hospitals E.E. Stobberingh A1, J. Arends A2, J.A.A. Hoggkamp-Korstanje A3, W.H.F. Goessens A4, M.R. Visser A5, A.G.M. Buiting A6, Y.J. Debets-Ossenkopp A7, R.J. van Ketel A8, M.L. van Ogtrop A9, L.J.M. Sabbe A10, G.P. Voorn A9, H.L.J.

Winter A11, J.H. van Zeijl A12 A1 University Hospital Maastricht, Medical Microbiology, P.O. Box 5800, NL-6202 AZ Maastricht, The Netherlands.

121. Journal of Clinical Microbiology, June 1999, p. 1758-1763, Vol. 37, No. 60095-1137/99/$04.00+0 Survey of Extended-Spectrum -Lactamases in Clinical Isolates of Escherichia coli and Klebsiella pneumoniae: Prevalence of TEM-52 in Korea Hyunjoo Pai,1,* Sen Lyu,1 Ji Hyang Lee,1 Jungmin Kim,2 Youngmi Kwon,2 Jong-Won Kim,3 and Kang Won Choe4.

122. Journal of Clinical Microbiology, May 1998, p. 1446-1449, Vol. 36, No. 5 0095-1137/98/$04.00+0 Survey of Klebsiella pneumoniae Strains Producing Extended-Spectrum -Lactamases: Prevalence of SHV-12 and SHV-2a in Korea Jungmin Kim,1,* Youngmi Kwon,1 Hyunjoo Pai,2 Jong-Won Kim,3 and Dong-Taek Cho4 Department of Microbiology1 and Department of Internal Medicine,2 College of Medicine, Dankook University, Chonan, Department of Clinical Pathology, College of Medicine, Sungkyunkwan University, Seoul,3 and Department of Microbiology, College of Medicine, Kyungpook University, Taegu,4 Korea.

123. Journal of Antimicrobial Chemotherapy (1992) 30, 429-447 © 1992 The British Society for Antimicrobial Chemotherapy Survey of the prevalence of ßlactamases amongst 1000 Gram-negative bacilli isolated consecutively at the Royal London Hospital P. Y. F. Liu, Deniz Gur, Lucinda M. C. Hall and D. M. Livermore* Department of Medical Microbiology, The London Hospital Medical College Turner Street, London E1 2AD, UK.

124. Antimicrobial Agents and Chemotherapy, August 1999, p. 2069-2073, Vol. 43, No. 8 0066-4804/99/$04.00+0 TEM-24 Produced by Four Different Species of Enterobacteriaceae, Including Providencia rettgeri, in a Single Patient Helene Marchandin,1,* Christian Carriere,1 Danielle Sirot,2 Helene Jean-Pierre,1 and Helene Darbas1.

125. Antimicrobial Agents and Chemotherapy, September 2000, p. 2537-2539, Vol. 44, No. 9 0066-4804/00/$04.00+0 TEM-72, a New Extended-Spectrum -Lactamase Detected in Proteus mirabilis and Morganella morganii in Italy Mariagrazia Perilli,1 Bernardetta Segatore,1 Maria Rosaria De Massis,1 Maria Letizia Riccio,2 Ciro Bianchi,3 Alessandro Zollo,3 Gian Maria Rossolini,2 and Gianfranco Amicosante1,*.

126. Clinical Infectious Disease - Published by the University of Chicago Press Control of a Prolonged Outbreak of Extended-Spectrum β-Lactamase–Producing Enterobacteriaceae in a University Hospital Jean-Christophe Lucet, Dominique Decré, Anika Fichelle, Marie-Laure Joly-Guillou, Micheline Pernet, Claude Deblangy, Marie-Jeanne Kosmann, and Bernard Régnier Clinical Infectious Diseases, volume 29 (1999), pages 1411–1418 DOI: 10.1086/313511 PubMed ID: 10585788.

127. Clinical Infectious Disease - Published by the University of Chicago Press Epidemiology of Ciprofloxacin Resistance and Its Relationship to Extended-Spectrum β-Lactamase Production in Klebsiella pneumoniae Isolates Causing Bacteremia David L. Paterson, Lutfiye Mulazimoglu, Jose Maria Casellas, Wen-Chien Ko, Herman Goossens, Anne Von Gottberg, Sunita Mohapatra,

Gordon M. Trenholme, Keith P. Klugman, Joseph G. McCormack, and Victor L. Yu Clinical Infectious Diseases, volume 30 (2000), pages 473–478.

128. Antimicrobial Agents and Chemotherapy, April 2000, p. 997-1003, Vol. 44, No. 4 0066-4804/00/$04.00+0 TLA-1: a New Plasmid-Mediated Extended-Spectrum -Lactamase from Escherichia coli J. Silva,1,* C. Aguilar,1, G. Ayala,2 M. A. Estrada,1 U. Garza-Ramos,1 R. Lara-Lemus,2 and L. Ledezma1.

129. New Microbiol. 2005 Jul;28(3):231-6. An outbreak of SHV-5 producing Klebsiella pneumoniae in a neonatal intensive care unit; meropenem failed to avoid fecal colonization. Gundes S, Arisoy AE, Kolayli F, Karaali E, Turker G, Sanic A, Arisoy ES, Vahaboglu H. Infeksiyon Hastaliklari & Klinik Mikrobiyoloji AD, Kocaeli Universitesi, Kocaeli, Turkey.

130. Antimicrob Agents Chemother. 1990 Nov;34(11):2210-6. Development of "oligotyping" for characterization and molecular epidemiology of TEM beta-lactamases in members of the family Enterobacteriaceae. Mabilat C, Courvalin P. Unite des Agents Antibacteriens, Institut Pasteur, Paris, France.

aPPENDIX

APPENDIX

Media Composition

Mueller Hinton Agar : this medium is more commonly used in conjunction with high potency antibiotic risk for the determination of antibiotic sensitivity patterns by the Kirby-Bauer technique :

Beef infusion	-	300 ml
Caesin hydrolysate	-	17.5 g
Starch	-	1.5 g
Agar	-	10 g
Distilled Water	-	1 Ltr.

Emulsify Starch in a small amount of cold water pour into the beef infusion and add the Caesin hydrolysate and the Agar. Make up the volume to 1 liter with distilled water. Dissolve the constituents by heating gently at 100^{o}C with agitation. Filter if necessary. Adjust the pH to 7.4. Dispense screw capped bottle and sterilize by autoclaving at 121^{o}C for 20 minutes.

MacConkey Agar : Useful medium for the cultivation of Enterobacteria

Peptone	-	20 g
Sodium taurocholate Commercial	-	5 g
Water	-	1 Ltr.
Agar	-	20 gm

Neutral Red Solution - 2% in 50% ethanol = C 3.5 ml = Lactose 10% aqueous solution = 100ml. Dissolve Peptone and taurocholate (Bile salt) in the water by heating. Add the Agar and dissolve it in the steamer or autoclave. Adjust the pH to 7.5. Add the lactose and neutral red and mix. Heat in the autoclave with free steam (100^{o}C) for 1 hour then 115^{o}C for 15 minutes. Pour Plates.

Blood Agar : The BDM is prepared by adding sterile blood to sterile nutrient agar that has been melted and cold to 50^{o}C. 10% is the most usual concentration of blood. Horse blood is the commonest. Double layer blood agar is usually the best. A thin layer of melted nutrient agar about 7 ml for a 9cm pertidish is poured and allowed to set. Then a similar thin layer of 10% blood agar is poured on top of the first layer.

Nutrient Agar : Is nutrient brought solidified by the addition of agar.

McFarland Standard : Preparation - McFarland Standard is 0.5 barium sulphate standard. Standard is prepared by adding 0.5ml of (w/v 1.17% $Bacl_2$ Water) to 99.5 ml of (1% v/v H_2So_4). Aliquots of 4-6 ml of barium sulphate standard are distributed in screw capped bottles. Sealed and can be stored for six months in dark room and room temperature (20-28^{o}C).

Gram Staining : Reagents :

Primary Stain

Crystal Violet	-	10 g
Absolute alcohol	-	100ml
Distilled water	-	1 Ltr.

Mordant

Iodine	-	10 g
Potassium Iodide	-	20 g
Distilled water	-	1 Ltr.

Decolouriser - Acetone Alcohol Mixture

Acetone	-	50ml
95% Ethanol	-	50 ml

Counter stain

Carbol Fuschin Strong	-	100ml
Distilled Water	-	1 Ltr.

Specimen Processing : The specimens were processed as per standard protocol for isolation of bacteria.

Smear preparation : Grams Smear –

1. The clinical material was spread thinly and uniformly over a clean glass slide.
2. Smears from culture on solid media were prepared by emulsifying the colonies in a drop of saline placed on the slide and spread over thinly.
3. Smear was allowed to dry in air and then heat fixed by gently passing over a flame once or thrice.

Gram Staining

1. The heat fixed smear was flooded with methyl violet kept for 60 seconds.

2. The stain was poured off. Then the smear was washed with sterile distilled water covered with gram iodine for 60 seconds.
3. Again the smear was washed with distilled water and decolourised with acetone alcohol mixture for not more than 10 seconds.
4. The smear was washed with distilled water and counter stained with carbol fuschin for 30 seconds.
5. Then the smear was washed again with distilled water, air-dried and observation was made under oil immersion (100x).